

AR201-14066B

Substance Group: Group 5 - Zinc Dialkyldithiophosphates

Summary Prepared by: Petroleum Additives Panel
Health & Environmental Research Task Group

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1.0 Biodegradation

Robust Summary 5-BioDeg-1

<u>Test Substance</u>	
CAS #	54261-67-5
Chemical Name	Phenol, dodecyl-, hydrogen phosphorodithioate zinc salt
Remarks	This substance is referred to as ----- in the HERTG's Test Plan for Zinc Alkyl Dithiophosphate Category. For more information on the chemical, see Section 2.0 "Chemical Description of Zinc Alkyl Dithiophosphate Category" in HERTG's Test Plan for Zinc Alkyl Dithiophosphate Category.
<u>Method</u>	
Method/Guideline followed	OECD Method 310B; U.S. EPA Method 796.3260; ASTM D5864-95.
Test Type (aerobic/anaerobic)	Aerobic
GLP (Y/N)	N
Year (Study Performed)	1998
Contact time (units)	28 days.
Inoculum	Activated sludge supernatant from domestic wastewater treatment plant and soil filtrate.
Remarks for test conditions	<p><u>Inoculum</u>: Soil was collected from a wooded lot to a depth of 20 cm (surface soil was not included). Prior to use, 200 gm (wet weight) of soil was suspended in 2 L of water, allowed to settle for 30 min and filtered through glass wool. Filtrate was aerated until use. Activated sludge from domestic wastewater treatment plant was sieved through a 2 mm screen, aerated for 4 hours and homogenized in a blender. The sludge was allowed to settle for 30 minutes and supernatant was removed for use.</p> <p><u>Concentration of test chemical</u>: Sufficient amount of test material was added to each flask, giving 10 mg C/L in the test flasks.</p> <p><u>Temp of incubation</u>: 20 ± 3°C.</p> <p><u>Dosing procedure</u>: No organic solvents were used to facilitate dissolution of the test material. Test material addition was added directly to the treatment group chamber to achieve the final volume.</p> <p><u>Test Setup</u>: Total volume of liquid in test chambers was 3 L. The biodegradation test was started by bubbling CO₂ free air through the test media at a rate of 50 to 100 mL per minute. The CO₂ generated within each test chamber was trapped as K₂CO₃ in the</p>

	<p>KOH solution and measured using a carbon analyzer.</p> <p><u>Sampling frequency:</u> CO₂ traps were removed for analysis on Days 2, 5, 11, 13, 16, 18, 23, and 29. On day 28, the test was terminated by the acidification of the test chamber to release dissolved CO₂.</p> <p><u>Controls:</u> Blank and positive controls were included; abiotic and toxicity controls were not. Canola oil was used as the reference substance in the positive controls.</p> <p><u>Analytical method:</u> KOH ("trap") solutions were used downstream of the test flasks to trap generated CO₂ as K₂CO₃. The CO₂ produced was measured using a carbon analyzer.</p> <p><u>Method of calculating measured concentrations:</u> N/A</p> <p><u>Other:</u> A preadapted inoculum was used for the biodegradation test. Equal volumes of the activated sludge supernatant and soil filtrate were combined and supplemented with vitamin free casamino acids and 25 mg/L yeast extract. 100 ml of the supplemented inoculum was combined with 900 ml of test medium within each 2-L erlenmeyer flask. The solutions were aerated with CO₂ free air and test substances added incrementally at concentrations equivalent to 4, 8, and 8 mg C/L on days 0, 7, and 11, respectively. On day 14, an equal volume of each culture was combined and composite inoculum screened using glass wool and homogenized in a blender. Standard plate count prior to the 14-day adaptation period was 1.36×10^5 CFU/mL. Standard plate count after 14-day adaptation was 2.62×10^5 CFU/mL.</p>
<u>Results</u>	
Test Substance Degradation, % after time	5.9% after 28 days
Kinetic (for sample, positive and negative controls)	Reference (Canola oil): 92% (2 - 5 day lag period) Test substance: 5.9% (28d; 11 – 16 day lag period)
Breakdown Products (Y/N) If yes describe breakdown products	N
Remarks	
<u>Conclusions</u>	Test substance degraded 5.9% in 28 days. The reference substance, canola oil, degraded 92% in the same test period.
<u>Data Quality</u>	(1) Reliable without restrictions.
<u>References</u>	This robust summary was prepared from an unpublished study by an individual member company of the HERTG (the underlying study contains confidential business information).
<u>Other</u>	Updated: 11-19-2000

Robust Summary 5-BioDeg-2

Test Substance	
CAS #	54261-67-5
Chemical Name	Phenol, dodecyl-, hydrogen phosphorodithioate zinc salt
Remarks	This substance is referred to as ----- in the HERTG's Test Plan for Zinc Alkyl Dithiophosphate Category. For more information on the chemical, see Section 2.0 "Chemical Description of Zinc Alkyl Dithiophosphate Category" in HERTG's Test Plan for Zinc Alkyl Dithiophosphate Category.
<u>Method</u>	
Method/Guideline followed	OECD 301F
Test Type (aerobic/anaerobic)	Aerobic
GLP (Y/N)	Y
Year (Study Performed)	1998
Contact time (units)	28 days.
Inoculum	Activated sludge from domestic wastewater treatment plant.
Remarks for test conditions	<p><u>Inoculum</u>: The supernatant from the homogenized activated sludge was used as inoculum. The sludge was homogenized in a blender at medium speed for approximately 2 minutes and allowed to settle for approximately 30 minutes. The supernatant was used for inoculum preadaptation. Bacterial counts in the inocula were 1×10^5 to 2×10^6 cells/mL.</p> <p><u>Concentration of test chemical</u>: Approximately 100 mg/L of test material was added to the treatment group by direct weight addition.</p> <p><u>Temp of incubation</u>: $20 \pm 1^\circ\text{C}$.</p> <p><u>Dosing procedure</u>: No organic solvents were used to facilitate the dispersion of the test material. The substances were weighed onto a solid carrier (a small Teflon coupon) and introduced into the medium. The reference material was added directly.</p> <p><u>Test Setup</u>: 900 mL of the test media was mixed with 100 mL of the inoculum. The mixture was continuously stirred in a closed flask at a constant temperature for up to 28 days. A BI-1000 electrolytic respirometer system was used for this study. Each reactor flask is equipped with an electrolytic cell assembly, which generates oxygen to replace the amount consumed by the test mixture in the flask.</p> <p><u>Sampling frequency</u>: The oxygen uptake in all flasks were monitored continuously and recorded automatically every 4 hours throughout the test period using the BI Data Acquisition</p>

	<p>software.</p> <p><u>Controls:</u> Yes (blank and positive controls per guideline). Abiotic and toxicity checks were not included. Sodium benzoate was used as the positive control.</p> <p><u>Analytical method:</u> A BI-1000 respirometer was used to continuously monitor O₂ uptake. The O₂ uptake was recorded every 4 hours.</p> <p><u>Method of calculating measured concentrations:</u> N/A</p> <p><u>Other:</u> A preadapted inoculum was used for the biodegradation test. The activated sludge supernatant was combined and supplemented with vitamin free casamino acids and 25 mg/L yeast extract. 100 ml of the supplemented inoculum was combined with 900 ml of test medium within each 2-L erlenmeyer flask. The solutions were aerated with CO₂ free air and test substances added incrementally at concentrations equivalent to 4, 4, and 8 mg C/L on days 0, 7, and 12, respectively. On day 14, each adapted culture was homogenized in a blender and a composite culture prepared by mixing equal volumes of the homogenized cultures.</p>
<u>Results</u>	
Degradation % after time	4.2% after 28 days
Kinetic (for sample, positive and negative controls)	Reference (sodium benzoate) – 82.3% (28d). An average percent biodegradation of 60% was achieved within 3 days, thereby fulfilling the criteria for a valid test reaching the pass level by day 14. Test substance – 4.2% (28d)
Breakdown Products (Y/N) If yes describe breakdown products	N
<u>Remarks</u>	
<u>Conclusions</u>	Test material degraded 4.2% in 28 days. The reference substance, sodium benzoate, reached a level of 82.3% in the same test period.
<u>Data Quality</u>	(1) Reliable without restrictions.
<u>References</u>	This robust summary was prepared from an unpublished study by an individual member company of the HERTG (the underlying study contains confidential business information).
<u>Other</u>	Updated: 11-19-2000

Robust Summary 5-BioDeg-3

Test Substance	
CAS #	84605-29-8
Chemical Name	Phosphorodithioic acid, mixed O,O-bis (1,3-dimethylbutyl and iso-Pr) esters, zinc salts
Remarks	This substance is referred to as ----- in the HERTG's Test Plan for Zinc Alkyl Dithiophosphate Category. For more information on the chemical, see Section 2.0 "Chemical Description of Zinc Alkyl Dithiophosphate Category" in HERTG's Test Plan for Zinc Alkyl Dithiophosphate Category.
<u>Method</u>	
Method/Guideline followed	OECD Method 310B; & EEC Method C.4-C (2).
Test Type (aerobic/anaerobic)	Aerobic
GLP (Y/N)	Y
Year (Study Performed)	1998
Contact time (units)	28 days.
Inoculum	Activated sludge supernatant from domestic wastewater treatment plant and soil filtrate.
Remarks for test conditions	<p><u>Inoculum</u>: Activated sludge from domestic waste water treatment plant was sieved through a 2 mm screen, aerated for 4 hours and homogenized in a blender. The sludge was allowed to settle for 30 mins and supernatant was used as the inoculum the same day that it was prepared.</p> <p><u>Concentration of test chemical</u>: Sufficient amount of test material was added to each flask, giving 10 mg C/L in the test flasks.</p> <p><u>Temp of incubation</u>: 20 ± 2°C.</p> <p><u>Dosing procedure</u>: No organic solvents were used to facilitate dissolution of the test material. Test material addition was added directly to the treatment group chamber to achieve the final volume.</p> <p><u>Test Setup</u>: Total volume of liquid in test chambers was 3 L. The biodegradation test was started by bubbling CO₂ free air through the test media at a rate of 50 to 100 mL per minute. The CO₂ generated within each test chamber was trapped as K₂CO₃ in the KOH solution and measured using a carbon analyzer.</p> <p><u>Sampling frequency</u>: CO₂ traps were removed for analysis on Days 1, 4, 8, 11, 14, 19, 21, 25, and 29. The CO₂ trap nearest the chamber was removed. On day 28, the test was terminated by the acidification of the test chamber and aerated overnight to release dissolved CO₂. The trapping solutions closest to the test chambers were analyzed for inorganic carbon.</p>

	<p><u>Controls</u>: Blank and positive controls were included; abiotic and toxicity controls were not. Sodium benzoate was used as the reference substance in the positive controls.</p> <p><u>Analytical method</u>: KOH ("trap") solutions were used downstream of the test flasks to trap generated CO₂ as K₂CO₃. The CO₂ produced was measured using a carbon analyzer.</p> <p><u>Method of calculating measured concentrations</u>: N/A</p> <p><u>Other</u>: n/a</p>
<u>Results</u>	
Test Substance Degradation, % after time	5.9% after 28 days
Kinetic (for sample, positive and negative controls)	Reference (Sodium benzoate): 99.3%. An average percent biodegradation of 60% was achieved within 7 days, thereby fulfilling the criteria for a valid test reaching the pass level by day 14. Test substance: 1.5% (28d)
Breakdown Products (Y/N) If yes describe breakdown products	N
<u>Remarks</u>	
<u>Conclusions</u>	Test substance degraded 1.5% in 28 days. The reference substance, canola oil, degraded 99.3% in the same test period.
<u>Data Quality</u>	(1) Reliable without restrictions.
<u>References</u>	This robust summary was prepared from an unpublished study by an individual member company of the HERTG (the underlying study contains confidential business information).
<u>Other</u>	Updated: 11-19-2000

2. Toxicity

Category:

2.1 Acute Toxicity

2.1.1 Acute Oral Toxicity

Robust Summary 5-Acute Oral-1

<u>Test Substance</u>	
CAS #	CAS# 2215-35-2
Chemical Name	2-pentanol, 4-methyl-, hydrogen phosphorodithioate, Zn salt
Remarks	Test material dosed as received, purity not provided.
<u>Method</u>	
Method/Guideline followed	EPA FIFRA 81-1 (November 1982)
Test Type	Acute oral toxicity
GLP (Y/N)	Y
Year (Study Performed)	1986
Species/Strain	Rats/Sprague-Dawley strain
Sex	Male and Female
No. of animals/dose	5/sex
Vehicle	None
Route of administration	Oral (intragastric)
Dose level	2.0 and 5.0 g/kg
Dose volume	0.2 and 0.5 ml/100 g
Control group included	No
Remarks field for test conditions	A single dose of the undiluted test material was administered intragastrically to five fasted (over night) male and female rats at each treatment level. A control group was not included. The animals were observed for signs of toxicity or behavioral changes daily. Individual weights were recorded on the day of dosing, on day 7 and at termination. All animals were euthanized at the conclusion of the observation period. Gross autopsies were performed on all animals after 14 days.
<u>Results</u>	LD50 = Between 2.0 and 5.0 g/kg (males and females)
Remarks	All of the males and females at the 5 g/kg dose level died three to four days after dosing. Two low dose females died (days 1 and 4). All of the low dose males survived. Signs of toxicity at both dose levels included diarrhea, stained and/or ruffled fur and hypoactivity. Necropsy findings observed in the animals that died included gastrointestinal findings; bloody oral and nasal discharge and signs of diarrhea. There were no significant necropsy findings in the animals that survived to study termination.

<u>Conclusions</u>	The test article, when administered as received to male and female Sprague-Dawley rats, had an acute oral LD50 of between 2.0 and 5.0 g/kg (males and females.).
<u>Data Quality</u>	Reliable without restriction (Klimisch Code)
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 7/12/00 (RTA-042)

Robust Summary 5-Acute Oral-2

<u>Test Substance</u>	
CAS #	CAS# 4259-15-8
Chemical Name	
Remarks	Test material dosed as received, purity not provided.
<u>Method</u>	
Method/Guideline followed	FHSA 16CFR1500.3
Test Type	Acute oral toxicity
GLP (Y/N)	N
Year (Study Performed)	1975
Species/Strain	Rats/ Sprague-Dawley strain
Sex	Male
No. of animals/dose	10
Vehicle	None
Route of administration	Oral (intra gastric)
Dose level	0, 0, 2.2, 3.3, 5.0, and 7.5 g/kg
Dose volume	Not provided
Control group included	Yes
Remarks field for test conditions	A single dose of the undiluted test material was administered intragastrically to ten fasted male rats at each treatment level. Two control groups were included. The animals were observed for signs of toxicity or behavioral changes daily. Individual weights were recorded on the day of dosing, on day 7 and at termination. All animals were euthanized at the conclusion of the observation period. Gross autopsies were performed on all animals after 14 days.
<u>Results</u>	LD50 = 3.1 (1.8-5.1) g/kg (males)
Remarks	Mortality occurred in males dosed at all dose levels. Two of ten animals died at the low dose level. All animals died at the high dose. Signs of toxicity observed in all groups included diarrhea, depression, and reduced food consumption. Surviving animals exhibited recovery from these signs of toxicity between 3 and 12 days after dosing. At necropsy all treated animals exhibited a reduced amount of body fat. No other treatment related necropsy findings were observed.
<u>Conclusions</u>	The test article, when administered as received to male Sprague-Dawley rats, had an acute oral LD50 of 3.1 (1.8-5.1) g/kg.
<u>Data Quality</u>	Reliable with restriction (Klimisch Code). Restriction due to the fact that this is a summary report.
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 8/31/00 (RTA-041)

Robust Summary 5-Acute Oral-3

<u>Test Substance</u>	
CAS #	CAS# 68457-79-4
Chemical Name	Phosphorodithioic acid, mixed O,O-bis(iso-Bu and pentyl) esters, zinc salts
Remarks	Test material dosed as received, purity not provided.
<u>Method</u>	
Method/Guideline followed	FHSA 16CFR1500.3
Test Type	Acute oral toxicity
GLP (Y/N)	N
Year (Study Performed)	1979
Species/Strain	Rats/Wistar strain
Sex	Male
No. of animals/dose	10
Vehicle	None
Route of administration	Oral (intragastric)
Dose level	2.0, 3.5, 5.0 and 8.75 g/kg
Dose volume	2-10 ml/kg
Control group included	No
Remarks field for test conditions	A single dose of the undiluted test material was administered intragastrically to ten fasted (over night) male rats at each treatment level. A control group was not included. The animals were observed for signs of toxicity or behavioral changes daily. Individual weights were recorded on the day of dosing and at termination. All animals were euthanized at the conclusion of the observation period. Gross autopsies were performed on all animals after 14 days.
<u>Results</u>	LD50 = 3.6 (2.7-4.8) g/kg (males)
Remarks	All of the 8.75 g/kg animals died during the first two days of study. Eight of ten males treated at 5.0 g/kg died during days 1 through 3. Six males at the 3.5 g/kg dose level died between days 2 and 5. No deaths occurred at the 2.0 g/kg dose level. Lethargy, diarrhea, ptosis, chromorhinorrhea, piloerection, and chromodacryorrhea were noted in all groups. These findings were no longer evident in the two lowest groups by the middle of week two. Body weight changes were within expected ranges for the surviving animals. Lung congestion, gastrointestinal findings and staining around the mouth, nose and anus were common necropsy findings for the animals that died.
<u>Conclusions</u>	The test article, when administered as received to male Wistar rats, had an acute oral LD50 of 3.6 g/kg.
<u>Data Quality</u>	Reliable without restriction (Klimisch Code)
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 7/11/00 (RTA-038)

Robust Summary 5-Acute Oral-4

<u>Test Substance</u>	
CAS #	CAS# 68784-31-6
Chemical Name	Phosphorodithioic acid, mixed O,O-bis(sec-Bu and 1,3-dimethylbutyl) esters, zinc salts
Remarks	Test material dosed as received, purity not provided.
<u>Method</u>	
Method/Guideline followed	OECD 401
Test Type	Acute oral toxicity
GLP (Y/N)	Y
Year (Study Performed)	1981
Species/Strain	Rats/ Sprague-Dawley strain
Sex	Male and Female
No. of animals/dose	5/sex
Vehicle	None
Route of administration	Oral (intragastric)
Dose level	0, 1.8, 2.7, 4.0 and 6.0 g/kg
Dose volume	0.4-1.6 ml/kg
Control group included	Yes
Remarks field for test conditions	A single dose of the undiluted test material was administered intragastrically to five fasted (over night) male and female rats at each treatment level. A control group was included. The animals were observed for signs of toxicity or behavioral changes daily. Individual weights were recorded on the day of dosing, on day 7 and at termination. All animals were euthanized at the conclusion of the observation period. Gross autopsies were performed on all animals after 14 days.
<u>Results</u>	LD50 = 3.4 (2.2-5.2) g/kg (males); 2.9 (1.9-4.7) g/kg (females)
Remarks	Mortality occurred in males dosed with 4.0 g/kg and greater and in females dosed with 2.7 g/kg and greater. Signs of toxicity included diarrhea, depression, reduced food consumption, weakness, salivation, blood in the urine and death. Body weights in the 2.7 g/kg and greater males and/or females were significantly less than control at seven days. No treatment related necropsy findings were observed.
<u>Conclusions</u>	The test article, when administered as received to male and female Sprague-Dawley rats, had an acute oral LD50 of 3.4 (2.2-5.2) g/kg (males) and 2.9 (1.9-4.7) g/kg (females).
<u>Data Quality</u>	Reliable without restriction (Klimisch Code)
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 7/11/00 (RTA-040)

Robust Summary 5-Acute Oral-5

<u>Test Substance</u>	
CAS #	CAS# 84605-29-8
Chemical Name	Phosphorodithioic acid, mixed O,O-bis (1,3-dimethylbutyl and iso-Pr) esters, zinc salts
Remarks	Test material dosed as received, purity not provided.
<u>Method</u>	
Method/Guideline followed	OECD 401
Test Type	Acute oral toxicity
GLP (Y/N)	Y
Year (Study Performed)	1980
Species/Strain	Rats/ Sprague-Dawley strain
Sex	Male and Female
No. of animals/dose	10/sex
Vehicle	Corn Oil
Route of administration	Oral (intra-gastric)
Dose level	1500, 1825, 2221, 2702, 3288 and 4000 mg/kg
Dose volume	15 ml/kg
Control group included	No
Remarks field for test conditions	A single dose of the undiluted test material was administered intra-gastrically to ten fasted (over night) male and female rats at each treatment level. (Thirteen females were inadvertently dosed in Group 3 and seven females were dosed in Group 4.) A control group was not included. The animals were observed for signs of toxicity or behavioral changes daily. Individual weights were recorded on the day of dosing, and at termination. All animals were euthanized at the conclusion of the observation period. Gross autopsies were performed on all animals after 14 days.
<u>Results</u>	LD50 = 3.2 (2.6-4.0) g/kg (males); 3.1 (2.6-3.8) g/kg (females)
Remarks	Deaths occurred in all groups greater than 1825 mg/kg within 93 hours of dosing. Signs of toxicity included hypokinesia at all dose levels, ataxia at doses above 1825 mg/kg and diarrhea in almost all animals in all dose groups. All surviving animals gained weight. Necropsy findings in animals that died on test were limited to findings suggestive of post mortem changes. No treatment related necropsy findings were observed. Surviving animals had no remarkable necropsy findings.
<u>Conclusions</u>	The test article, when administered as received in corn oil to male and female Sprague-Dawley rats, had an acute oral LD50 of 3.2 (2.6-4.0) g/kg (males); 3.1 (2.6-3.8) g/kg (females).
<u>Data Quality</u>	Reliable without restriction (Klimisch Code)
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 7/12/00 (RTA-043)

2.1.2 Acute Dermal Toxicity

Robust Summary 5-Acute Dermal-1

<u>Test Substance</u>	
CAS #	CAS# 4259-15-8
Chemical Name	
Remarks	Test material dosed as received, purity not provided.
<u>Method</u>	
Method/Guideline followed	Similar to OECD Guideline 402
Test Type	Acute dermal toxicity (Limit Test)
GLP (Y/N)	N
Year (Study Performed)	1975
Species/Strain	Rabbits/New Zealand White
Sex	Male
No. of animals/group	6
Vehicle	None
Route of administration	Dermal
Dose level	0 and 5 g/kg
Dose volume	Not provided.
Control group included	Yes
Remarks field for test conditions	<p>This study was conducted prior to the development of Test Guideline 402. This study deviated from Guideline 402 in that the skin of 3 treated animals were abraded prior to dosing. In addition the guideline calls for the evaluation of males and females using at least one dose level. This study was conducted using males only. These deviations were not considered sufficient to change the outcome of the study.</p> <p>Approximately 24 hours prior to topical application of the test material, the hair of each animal was closely clipped. On the day of dosing the skin of three treated animals was abraded prior to test material administration. A single dose of 5 g/kg of the undiluted test material was administered dermally to six male animals. The test material was kept in contact with the skin for a period of 24 consecutive hours under a gauze bandage covered with an elastic sheet. The application site was wiped clean of residual test material at the end of the 24-hour exposure period. All rabbits were fitted with collars for four days to prevent ingestion of the test material. The animals were observed for 14 days after treatment. Irritation was scored at 24, 48 and 72 hours and at 7 days post treatment. The surviving animals were euthanized at the conclusion of the observation period. Gross necropsies were performed on all animals on Day 14. The liver and lungs were examined microscopically.</p>
<u>Results</u>	LD50 > 5.0 g/kg (males)
Remarks	One abraded rabbit died 13 days after dosing. Reduced food consumption was observed in most animals during the last 10 days of

	test. All animals appeared depressed during the last week of study. Muscular weakness was observed in the abraded animals during the last three days of study. Sever erythema and edema were noted in the treated skin at 24 hours post dosing. At seven days the treated skin was thick and escharotic. All treated animals lost weight during the study. Control animals gained weight. At necropsy dermal findings were consistent with the in life evaluation. All animals had reduced amounts of body fat. Microscopic examination of gross lesions observed in the liver and lungs confirmed the presence of bronchopneumonia or chronic interstitial pneumonia and liver parasites. The lungs and livers of the control animals were unremarkable.
<u>Conclusions</u>	The test article, when administered dermally as received to 6 male New Zealand white rabbits had an acute dermal LD50 of greater than 5.0 g/kg.
<u>Data Quality</u>	Reliable with restriction (Klimisch Code). Restriction due to the fact that this is a summary report.
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 8/31/00 (RTA-044)

Robust Summary 5-Acute Dermal-2

<u>Test Substance</u>	
CAS #	CAS# 68457-79-4
Chemical Name	Phosphorodithioic acid, mixed O,O-bis(iso-Bu and pentyl) esters, zinc salts
Remarks	Test material dosed as received, purity not provided.
<u>Method</u>	
Method/Guideline followed	Similar to OECD Guideline 402
Test Type	Acute dermal toxicity (Limit Test)
GLP (Y/N)	N
Year (Study Performed)	1975
Species/Strain	Rabbits/New Zealand White
Sex	Male and Female
No. of animals/group	2/sex
Vehicle	None
Route of administration	Dermal
Dose level	20 g/kg
Dose volume	Not provided.
Control group included	No
Remarks field for test conditions	<p>This study was conducted prior to the development of Test Guideline 402. This study deviated from Guideline 402 in that 2 rather than 5 animal/sex were evaluated for toxicity. In addition the skin of one animal/sex was abraded prior to dosing. The guideline does not call for abraded skin. While the guideline calls for gross necropsies to be conducted on all animals they were not conducted during this study. Given the high dose level tested during this study and the lack of any mortality, these deviations were not considered sufficient to disqualify this study.</p> <p>Approximately 24 hours prior to topical application of the test material, the hair of each animal was closely clipped. On the day of dosing the skin of two treated animals was abraded prior to test material administration. A single dose of 20 g/kg of the undiluted test material was administered dermally to two male and two female animals. The test material was kept in contact with the skin for a period of 24 consecutive hours under a gauze bandage covered with an elastic sheet. The application site was wiped clean of residual test material at the end of the 24-hour exposure period. The animals were observed for 14 days after treatment. Irritation was scored at 24 hours post treatment using the Draize method. The surviving animals were euthanized at the conclusion of the observation period. Gross necropsies were not performed.</p>
<u>Results</u>	LD50 > 20.0 g/kg (males and female)
Remarks	All animals survived the study. Well defined erythema and slight edema were noted at 24 hours post dosing. Clinical signs including lethargy, diarrhea, ataxia, ptosis, alopecia, emaciation, nasal discharge

	and sensitivity to touch were noted during the second week of observation. All treated animals lost weight during the study.
<u>Conclusions</u>	The test article, when administered dermally as received to 2 male and 2 female New Zealand white rabbits had an acute dermal LD50 of greater than 20.0 g/kg.
<u>Data Quality</u>	Reliable with restriction (Klimisch Code). Restriction due to the fact that the study design differs significantly from the referenced guideline. However given the high dose level tested (20 g/kg) and the lack of mortality the study was considered valid and appropriate for review.
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 8/31/00 (RTA-045)

Robust Summary 5-Acute Dermal-3

<u>Test Substance</u>	
CAS #	CAS# 68784-31-6
Chemical Name	Phosphorodithioic acid, mixed O,O-bis(sec-Bu and 1,3-dimethylbutyl) esters, zinc salts
Remarks	Test material purity not provided
<u>Method</u>	
Method/Guideline followed	OECD Guideline 402
Test Type	Acute dermal toxicity (Limit Test)
GLP (Y/N)	Y
Year (Study Performed)	1981
Species/Strain	Rabbits/New Zealand White
Sex	Male and female
No. of animals/sex/group	5
Vehicle	None
Route of administration	Dermal
Dose level	5 g/kg
Dose volume	5 ml/kg
Control group included	Yes
Remarks field for test conditions	<p>This study deviates from the above referenced guideline in that the dosing site was abraded prior to treatment. This was not considered a significant deviation from the guideline that would adversely effect the study results.</p> <p>Approximately 24 hours prior to topical application of the test material, the hair of each animal was closely clipped. Immediately prior to dosing the skin was abraded. A single dose of 5 g/kg of the undiluted test material was administered dermally to five male and female animals. The test material was kept in contact with the skin for a period of 24 consecutive hours under an elastic bandage. The application site was wiped clean of residual test material at the end of the 24-hour exposure period. Collars were placed on the animals for six days to prevent ingestion of the test material. The animals were observed for abnormal clinical signs daily for 14 days after treatment. Individual body weights were recorded on the day of dosing and on days 7 and 14. Gross necropsies were performed on all animals on Day 14. A section of treated skin was examined from each animal microscopically.</p>
<u>Results</u>	LD50 > 5.0 g/kg (males and females)
Remarks	No mortality was observed. Clinical signs observed were reduced food consumption and decreased motor activity. Body weights of the treated males and females were significantly less than control on days 7 and 14. Microscopic examination of the treated skin showed diffuse subacute dermatitis. No gross necropsy effects other than skin effects were evident.

<u>Conclusions</u>	The test article, when administered dermally as received to 5 male and 5 female New Zealand white rabbits had an acute dermal LD50 of greater than 5.0 g/kg.
<u>Data Quality</u>	Reliable with restriction (Klimisch Code). Restriction due to the fact that most data are not presented in the report. Summary statements are provided.
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 7/13/00 (RTA-049)

Robust Summary 5-Acute Dermal-4

<u>Test Substance</u>	
CAS #	CAS# 68988-46-5
Chemical Name	Phosphorodithioic acid mixed o,o-bis(iso-Bu,iso-octyl, and pentyl) esters zinc salts
Remarks	Test material purity not provided
<u>Method</u>	
Method/Guideline followed	OECD Guideline 402
Test Type	Acute dermal toxicity (Limit Test)
GLP (Y/N)	N
Year (Study Performed)	1984
Species/Strain	Rabbits/New Zealand White
Sex	Male and female
No. of animals/sex/group	5
Vehicle	None
Route of administration	Dermal
Dose level	2 g/kg
Dose volume	2 ml/kg
Control group included	No
Remarks field for test conditions	<p>This study deviates from the above referenced guideline in that the dosing site was abraded prior to treatment. This was not considered a significant deviation from the guideline.</p> <p>Approximately 24 hours prior to topical application of the test material, the hair of each animal was closely clipped. Immediately prior to dosing the skin was abraded. A single dose of 2 g/kg of the undiluted test material was administered dermally to five male and female animals. The test material was kept in contact with the skin for a period of 24 consecutive hours under a gauze and elastic bandage. The application site was wiped clean of residual test material at the end of the 24-hour exposure period. The animals were observed for abnormal clinical signs frequently on the day of dosing and once daily for 14 days after treatment. Individual body weights were recorded on the day of dosing. The surviving animals were euthanized at the conclusion of the observation period. Gross necropsies were performed on all animals on Day 14.</p>
<u>Results</u>	LD50 > 2.0 g/kg (males and females)
Remarks	No mortality was observed. Clinical signs observed in all animals included cyanosis and decreased motor activity. The majority of animals exhibited motor incoordination. Four animals exhibited a loss of righting reflex. Recovery from most of these signs occurred by day three post treatment. Dermal findings included necrosis, edema and ulceration. Dermal irritation persisted through study termination. Gross pathological findings were limited to pitted kidneys in one female.

<u>Conclusions</u>	The test article, when administered dermally as received to 5 male and 5 female New Zealand white rabbits had an acute dermal LD50 of greater than 2.0 g/kg.
<u>Data Quality</u>	Reliable without restriction (Klimisch Code)
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 7/13/00 (RTA-048)

Robust Summary 5-Acute Dermal-5

<u>Test Substance</u>	
CAS #	CAS# 84605-29-8
Chemical Name	Phosphorodithioic acid, mixed O,O-bis (1,3-dimethylbutyl and iso-Pr) esters, zinc salts
Remarks	Test material purity not provided.
<u>Method</u>	
Method/Guideline followed	None
Test Type	Acute dermal toxicity (Limit Test)
GLP (Y/N)	N
Year (Study Performed)	1980
Species/Strain	Rabbits/New Zealand White
Sex	Male and female
No. of animals/sex/group	2
Vehicle	None
Route of administration	Dermal
Dose level	2 g/kg
Dose volume	2.2 ml/kg
Control group included	No
Remarks field for test conditions	Approximately 24 hours prior to topical application of the test material, the hair of each animal was closely clipped. On the day of dosing the skin was abraded prior to test material administration. A single dose of 2 g/kg of the undiluted test material was administered dermally to two male and two female animals. The test material was kept in contact with the skin for a period of 24 consecutive hours under a gauze patch and plastic bandage. The application site was wiped clean of residual test material at the end of the 24-hour exposure period. The animals were observed for abnormal clinical signs daily for 14 days after treatment. Individual body weights were recorded on the day of dosing. The surviving animals were euthanized at the conclusion of the observation period. Gross necropsies were performed on all animals on Day 14.
<u>Results</u>	LD50 > 2.0 g/kg (males and females)
Remarks	No mortality was observed during the study. Erythema was observed in one animal on days 2 and 3 post dosing. All animals exhibited peeling of the skin at the dose site on the last three days of study. One rabbit had pale kidneys at necropsy. All of the remaining animals were unremarkable.
<u>Conclusions</u>	The test article, when administered dermally as received to 2 male and 2 female New Zealand white rabbits had an acute dermal LD50 of greater than 2.0 g/kg. No evidence of systemic toxicity was observed.
<u>Data Quality</u>	Reliable without restriction (Klimisch Code)
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 7/13/00 (RTA-047)

Robust Summary 5-Acute Dermal-6

<u>Test Substance</u>	
CAS #	CAS# 25103-54-2
Chemical Name	isodecanol, hydrogen phosphorodithioate, zinc salt
Remarks	Test material purity not provided
<u>Method</u>	
Method/Guideline followed	OECD Guideline 402
Test Type	Acute dermal toxicity (Limit Test)
GLP (Y/N)	Y
Year (Study Performed)	1986
Species/Strain	Rabbits/New Zealand White
Sex	Male and female
No. of animals/sex/group	5
Vehicle	None
Route of administration	Dermal
Dose level	8 g/kg
Specific Gravity	1.06 g/ml
Control group included	No
Remarks field for test conditions	<p>This study deviates from the above referenced guideline in that the dosing site was abraded prior to treatment. This was not considered a significant deviation from the guideline that would adversely affect the study results.</p> <p>A range finding study, conducted at dose levels of 1, 3.2, 6.3 and 8 g/kg in one animal/sex/dose level preceded this study. Based on the range find study results, a limit test was conducted at the 8 g/kg dose level.</p> <p>Approximately 24 hours prior to topical application of the test material, the hair of each animal was closely clipped. Immediately prior to dosing the skin was abraded. A single dose of 8 g/kg of the undiluted test material was administered dermally to five male and female animals. The test material was kept in contact with the skin for a period of 24 consecutive hours under a gauze bandage covered by a rubber dam and an elastic bandage. The animals were observed for abnormal clinical signs at 0.5, 2 and 4 hours after the 24-hour period of exposure and twice daily thereafter for 14 days after treatment. Individual body weights were recorded on the day of dosing and on days 7 and 14. Gross necropsies were performed on all animals on Day 14.</p>
<u>Results</u>	LD50 > 8.0 g/kg (males and females)
Remarks	No mortality was observed. Clinical signs observed included moderate erythema and edema, decreased activity, decreased muscle tone, abnormal gait and abnormal stance. Fissuring and sloughing of the skin at the application site were also observed. Terminal necropsy revealed several white nodules throughout the lungs and a discoloration of the abdominal wall in one animal.

<u>Conclusions</u>	The test article, when administered dermally as received to 5 abraded male and 5 abraded female New Zealand white rabbits had an acute dermal LD50 of greater than 8.0 g/kg.
<u>Data Quality</u>	Reliable with restriction (Klimisch Code). Restriction due to the fact that most data are not presented in the report. Summary statements are provided.
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 12/14/01

2.2 Repeated Dose Toxicity

Robust Summary 5-Repeated Tox-1

<u>Test Substance</u>	
CAS #	CAS# 26566-95-0
Chemical Name	Phosphorodithioic acid, mixed 0-(2-ethylhexyl)- O-(2-methylpropyl) esters, zinc salts
Remarks	Test material purity not provided.
<u>Method</u>	
Method/Guideline followed	None
Test Type	3 week dermal toxicity study in rabbits
GLP (Y/N)	N
Year (Study Performed)	1979
Species	Rabbit
Strain	New Zealand White
Route of administration	Dermal, 5 days/week, to the clipped, unabraided, dorsal surface.
Duration of test	15 days of treatment
Doses/concentration levels	0, 0.21, 0.43 and 0.86 g/kg
Vehicle control	None
Dose volume	0.2, 0.4, 0.6 mL/kg/day
Sex	Males/Females
Frequency of treatment	Once/day, 5 days/week for a total of 15 doses.
control and treatment groups	5/sex/group
Post exposure observation period	None
Statistical methods	Means and standard deviations were reported
Remarks field for test conditions	The test material was applied to the clipped, unabraided dorsal surface of the rabbits 5 days/week for 3 weeks. Six dosing sites were used on each animal. Dosing sites were rotated daily. Elizabethan collars were used to prevent ingestion. Clinical observations were made twice daily. Dermal responses were evaluated daily, 24 hours post treatment (Draize). Body weight was recorded weekly during treatment. Hematology and clinical chemistry parameters were evaluated pretest and at termination of treatment. Macroscopic examinations were performed on all animals. A range of tissues was examined microscopically in all animals. The testes were examined a second time in 1980.
<u>Results</u>	
Remarks	One high dose male and one mid dose male and female died during the study. These animals exhibited a loss of body weight, lethargy, anorexia, adipsia emaciation and diarrhea prior to death. The surviving animals in the control and all treated groups exhibited various signs of distress including nasal and ocular discharge, and gastrointestinal findings. These findings increased in frequency over the course of the study. They were accompanied, in some cases, by lethargy and ptosis. Several animals exhibited anorexia and adipsia accompanied by emaciation. Dermal reactions were moderate in all of the treated animals through Week 1. Severe reactions were observed in all treated

	<p>animals by the ninth dose. All of the treated males and females at the mid and high dose lost weight during the treatment period. Leukocyte counts were increased slightly in all treated groups at termination. Necropsy findings included sporadic occurrences of dark lungs, broncho-pneumonia, intestinal findings, liver nodules, kidney discolorations and retroperitoneal lymphoma in one rabbit. No clear relationships to treatment were observed. Moderate to severe epithelial hyperplasia with surface exudate was observed in the treated skin of all treated rabbits. Severity was dose related. At the high dose ulceration was observed in five animals and necrosis was present in one. Four of five high dose and 2 of 5 mid dose males exhibited suppression of sperm formation. One control animal had severely reduced spermatogenesis. The spermatogenic findings between the control and high dose differ in that the high dose animals exhibit aspermia.</p>
<u>Conclusions</u>	<p>Based on the findings observed during this study this reviewer has concluded that an NOAEL was not established for this study. Significant toxicity was observed at all concentrations of the test material tested</p>
<u>Data Quality</u>	<p>Reliable with restriction (Klimisch Code). Restriction due to the fact that the individual testes findings are not presented for the re-evaluation. An explanation is not provided as to why the testes were reexamined nor as to why they were found to be normal at the time of the initial examination.</p>
<u>References</u>	<p>Unpublished confidential business information</p>
<u>Other</u>	<p>Updated: 7/21/00 (RTA-064)</p>

Robust Summary 5-Repeated Tox-2

<u>Test Substance</u>	
CAS #	CAS# 4259-15-8
Chemical Name	
Remarks	Test material purity not provided.
<u>Method</u>	
Method/Guideline followed	OECD 407
Test Type	28-day oral toxicity study in rats
GLP (Y/N)	Y
Year (Study Performed)	1993
Species	Rat
Strain	Sprague-Dawley CD, 57 (males)- 64 (females) days old at receipt
Route of administration	Oral gavage (syringe and dosing tube)
Duration of test	28 days of treatment
Doses/concentration levels (dose volume)	0, 10, 50, 125, 250 and 500 mg/kg/day (5ml/kg)
Vehicle	Corn oil (5 ml/kg)
Sex	Males and females
Exposure period	28-day treatment duration
Frequency of treatment	7 days/week
Number of animals/sex/group	5 rats/sex/group
Post exposure observation period	None
Statistical methods	Body weight, food consumption, organ weights and organ/body weight ratios were analyzed. Mean values of all dose groups were compared to control at each time interval. Tests included a one-way analysis of variance followed by Dunnett's test.
Dose rangefinding study	Yes (Five day repeated dose oral toxicity study)
Remarks field for test conditions	<p>Single oral doses were administered for 28 consecutive days using a gavage needle. Clinical observations were performed daily prior to dosing, at the time of dosing and approximately 1 hour following dosing. Viability checks were performed twice daily. Body weights were recorded every other day and prior to necropsy. Individual food consumption was measured weekly. Macroscopic examinations were performed on all animals. Select organs were weighed. A range of tissues was examined microscopically. These included the adrenals, esophagus, stomach, intestine, gonads, accessory sex organs and gross lesions from the control and two highest dose levels.</p> <p>Deviations from the OECD 407 test guidelines include:</p> <ul style="list-style-type: none"> • A functional observational battery for neurotoxicity was not performed since this test was not part of the OECD 407 guideline at the time the study was performed. • Hematology and clinical chemistry parameters were not evaluated. • Limited microscopic pathology was performed.

<u>Results</u>	
Remarks	<p>Three males and one female at 500 mg/kg/day died between study days 6 and 16. These deaths were considered treatment related. One female at 125 mg/kg/day died on day 7. The lack of any mortality at the higher dose level of 250 mg/kg suggests that this death was not treatment related. All other animals survived the duration of the study. Test article related clinical signs included changes in fecal consistency and coloration, staining of various body surfaces, rales, salivation and aggressive behavior in the 125, 250 and 500 mg/kg/day males and females. Rales and salivation were also observed in the 50 mg/kg/day males. Body weight gain was inhibited in the 250 mg/kg/day males and the 500 mg/kg/day males and females during the first two days of dosing. Reduced body weight gains were observed in the high dose males through study day 12. Food consumption was slightly reduced in the 500 mg/kg/day males and females during the first week of dosing. At necropsy a thickened mucosa of the nonglandular stomach was observed in one 500 mg/kg/day male and in one and two females at 250 and 500 mg/kg/day, respectively. Upon microscopic examination one 250 mg/kg/day male and all high dose females had submucosal edema on the glandular and/or non-glandular portions of the stomach. Three high dose females also had suppurative inflammation, primarily in the non-glandular portion of the stomach. These findings suggest a response to a gastric irritant. Mean absolute and relative adrenal weights in the 250 and 500 mg/kg/day males and females were increased. No histopathological lesions were associated with these increases.</p>
<u>Conclusions</u>	The Study Director concluded that the NOAEL for systemic toxicity was 10 mg/kg/day.
<u>Data Quality</u>	Reliable without restriction (Klimisch Code)
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 7/21/00 (RTA-062)

Robust Summary 5-Repeated Tox-3

<u>Test Substance</u>	
CAS #	CAS# 68988-46-5
Chemical Name	Phosphorodithioic acid mixed o,o-bis(iso-Bu, isooctyl, and pentyl) esters zinc salts
Remarks	Test material purity not provided.
<u>Method</u>	
Method/Guideline followed	OECD 410
Test Type	3 week dermal toxicity study in rabbits
GLP (Y/N)	N
Year (Study Performed)	1983
Species	Rabbit
Strain	New Zealand White
Route of administration	Dermal, 5 days/week, to the clipped, unabraded, dorsal surface.
Duration of test	15 days of treatment followed by a six week recovery period
Doses/concentration levels	Untreated control, sham control, vehicle control, 3 , 5 , 25 and 100%
Vehicle control	Base Oil Vehicle
Dose volume	2 mL/kg/day
Sex	Males
Frequency of treatment	Once/day, 5 days/week for a total of 15 doses.
Vehicle control and treatment groups	18rabbits/group. Eight of the initial 18 animals/group served as recovery animals. The test material was administered undiluted to the treated animal in the high dose group. The animals in the lower dose group received the test material diluted in the vehicle. Doses were administered based on individual animal body weights.
Post exposure observation period	6 weeks
Statistical methods	Body weight, hematology and clinical chemistry parameters, organ weights and organ/body weight ratios were analyzed. Mean values of all dose groups were compared pretreatment vs post treatment. Tests included ANOVA and Tukey's B Test.
Remarks field for test conditions	The test material was applied to the clipped, unabraded dorsal surface of the rabbits 5 days/week for 3 weeks. Elizabethan collars were used to prevent ingestion. (OECD Guideline 410 suggests the use of a gauze patch over the treatment site secured to the trunk with non-irritating tape and wrapped with an elastic sleeve. This procedure was not used during this study. This is considered a minor deviation from the Guideline.) Clinical observations were made 3 hours post dosing. Recovery animals were examined daily for the first week of recovery and once weekly thereafter. Dermal responses were evaluated weekly during treatment and immediately prior to sacrifice (Draize). Body weight was recorded weekly during treatment and recovery. (OECD Guideline 410 suggests the recording of food consumption. This parameter was not recorded during this study. This is considered a minor deviation from the guideline.) Hematology and clinical chemistry parameters were evaluated pretest and at termination of treatment and recovery. Macroscopic examinations were performed on all animals. Select organs were weighed. A range of tissues was examined microscopically in the control and low dose

	animals.
<u>Results</u>	
Remarks	<p>All animals treated at the 100% dose level, 15 of 18 in the 25% and 1 of 18 in the vehicle and 5% dose groups died or were sacrificed moribund. No deaths occurred in the 3%, sham or untreated control groups. During recovery three animals in the vehicle control and one at 5% were sacrificed moribund. The three surviving animals at the 25% dose level were sacrificed at termination of the treatment period.</p> <p>Erythema and edema were observed in all treated rabbits within 24 hours of the first application. Severity was proportional to test material concentration and duration of exposure. Severe erythema and /or eschar formation were observed at later scoring periods. Similar results were noted in the vehicle control animals. Other findings included hyperirritability, diarrhea, decreased motor activity, ataxia, loss of righting reflex, ocular discharge, redness in the genital area and rippling of skin.</p> <p>Severe body weight losses were seen at the 100% and 25% dose levels. Significant decreases in body weight of the vehicle control, 3% and 5% groups relative to untreated and/or sham control were noted throughout the study. Differences between vehicle control and the 3 and 5% dose groups were not statistically significant. Recovery from body weight losses began following termination of treatment with the vehicle or the test material.</p> <p>Significant reductions in several hematology parameters (RBC, HGB, HCT, MCH, MCHC) were noted in the vehicle and test material treated groups at the termination of treatment. Increases in the percentages of mature heterophiles and corresponding decreases in lymphocytes were detected.</p> <p>Mean absolute weights of testes, prostate and epididymis were significantly lower in the vehicle and test material treated groups than in the sham or untreated control groups. Differences in weight between vehicle and test material treated animals were not significant. Vehicle and test material related effects noted on the skin, hematology parameters, and weight of reproductive organs observed during treatment were no longer significant at the end of the 6-week recovery period.</p> <p>Gross necropsy findings observed in the vehicle and treated animals following the treatment period included discoloration of the dosing site, scaling, scabbing, hair loss, skin thickening. Treated animals also exhibited enlargement of the prefemoral lymph node, discoloration of the lungs and liver red foci in the gastric mucosa and trichobezoars in the stomach. One animal in each of the 5, 25% and vehicle control groups had small soft testes. Necropsy observations following recovery included less frequent and less severe skin lesions, enlargement of the prefemoral and respiratory lymph nodes and heart and lung and liver discoloration. In the vehicle control one rabbit had small prostate, seminal vesicles and coagulation glands. Two rabbits had mesenteric fat necrosis and three rabbits had small testes. In general vehicle treated animals had a higher incidence of lesions following recovery than did the treated animals.</p>

	<p>The skin of the vehicle and treated animals had acanthosis and acute and severe inflammation with pus formation at the treatment site. In the test material treated animals the acanthosis was followed by escharotic chemical dermatitis which involved the deeper layers of the skin and resulted in blood and fluid loss. Following the six-week recovery period the lesions of the epidermis had abated. A low incidence of orchitis and maturation arrest at the primary spermatocyte level was observed in the testes of vehicle and test material treated animals following the treatment and recovery periods. Atypical pneumonia and lymphadenopathy were also seen in these rabbits. These changes were also noted in the other study groups and therefore were not considered related to treatment.</p>
<u>Conclusions</u>	<p>Based on the findings observed during this study this reviewer has concluded that an NOAEL was not established for this study. Significant toxicity was observed at all concentrations of the test material tested. Effects noted at 3 and 5% of the test material were similar to those observed with the vehicle. Suggesting that toxic effects observed at these dose levels were principally due to the vehicle.</p>
<u>Data Quality</u>	<p>Reliable without restriction (Klimisch Code)</p>
<u>References</u>	<p>Unpublished confidential business information</p>
<u>Other</u>	<p>Updated: 7/21/00 (RTA-063)</p>

Robust Summary 5-Repeated Tox-4

<u>Test Substance</u>	
CAS #	113706-15-3
Chemical Name	Phosphorodithioic acid, secondary butyl and isooctyl) mixed esters, Zn Salts
Remarks	Test material purity not provided.
<u>Method</u>	
Method/Guideline followed	OECD 410
Test Type	28-day dermal toxicity study in rabbits
GLP (Y/N)	Y
Year (Study Performed)	1980
Species	Rabbit
Strain	New Zealand White (SPF)
Mean Weight at Initiation of Dosing	Males 2.1 kg. Females 2.3 kg.
Route of administration	Dermal, 5 days/week, to the clipped, unabraded, dorsal surface.
Duration of test	20 days of treatment
Doses/concentration levels	0, 5 and 25% (w/v) (OECD Guideline 410 suggests three treated groups and a control be included in this study design. The lowest dose level should be free of toxic effects. These suggestions were not met in this study. This was a two-treated group study. Effects were seen at the lowest dose level.)
Vehicle control	Yes (Primol 185)
Dose volume	2 mL/kg/day
Analytical confirmation of dose concentration	Samples were sent to the Sponsor for analysis.
Sex	Males and females
Frequency of treatment	Once/day, 5 days/week for a total of 20 doses.
Vehicle control and treatment groups	Ten male and ten female rabbits in the vehicle control group and in both treated groups. An untreated control group was not included in the study.
Post exposure observation period	None
Statistical methods	Body weight, hematology and clinical chemistry parameters, organ weights and organ/body weight ratios were analyzed. Mean values of all dose groups were compared to vehicle control at each time interval. Tests included parametric ANOVA with a Dunnett's test and regression analysis for linear response, non-parametric Kruskal-Wallis and Dunn's Summed Rank Test, Jonckheere's test for monotonic trend.
Remarks field for test conditions	The test material was applied to the clipped, unabraded dorsal surface of the rabbits for, 5 days/week for 20 days. Elizabethan collars were used to prevent ingestion. (OECD Guideline 410 suggests the use of a gauze patch over the treatment site secured to the trunk with non-irritating tape and wrapped with an elastic sleeve. This procedure was not used during this study. This is considered a minor deviation from the Guideline.) The backs of all animals were gently wiped with paper towels approximately 6 hours after exposure to remove excessive test substance, if necessary. Mortality and gross signs of toxicological and pharmacological effects were evaluated twice daily. Clinical examinations were made weekly. Dermal responses were evaluated daily (7 days/week). Body weight was recorded weekly during treatment. Hematology and clinical chemistry parameters were evaluated pretest and at termination of

	<p>treatment. Macroscopic examinations were performed on all animals. Select organs were weighed. Select tissues were examined microscopically for all animals in the vehicle control and high dose group. The testes were examined for all low dose males.</p>
<u>Results</u>	
Remarks	<p>Three high dose males and one high dose female were found dead or sacrificed moribund prior to study termination. These animals were found dead or were sacrificed on test days 8, 14, 17 and 18. One low dose males was found dead on test day 21. The cause of these deaths was not determined. All of the remaining vehicle control and treated animals survived the four-week duration of the study.</p> <p>Emaciation was a common finding in the 5% and 25% treated males and females. The incidence and severity of this finding were dose related. The high dose females were most affected. Dermal thickening was observed in all of the high dose animals during the last two weeks of study. Lacrimation was frequently observed in the high dose males and in the low and high dose females during the last two weeks of study. Lacrimation was observed in 2 vehicle control males and in 6 vehicle control females during the last week of the study. Ano-genital staining was observed in the high dose males and females.</p> <p>Low and high dose males and females generally exhibited marked, dose related increases in the incidence and severity of erythema, edema, atonia desquamation, fissuring, eschar formation and exfoliation. Many of these observations were moderate to extreme in severity, particularly those in the high dose group. Dermal irritation was observed in the vehicle control group. Severity and incidence were lower in the vehicle controls than in the test material treated animals.</p> <p>The mean body weights of the high dose males and females were reduced compared to vehicle control throughout the study. The high dose animals exhibited a loss in mean body weight over the treatment period. At study termination differences from vehicle control were –17% in the males and –20% in the females. The mean body weights of the low dose males and females were slightly reduced compared to vehicle control during the treatment period. Differences from vehicle control at termination were –2.9% in the males and –2% in the females.</p> <p>Mean hemoglobin, hematocrit and erythrocyte counts were statistically significantly reduced in the high dose males and females compared to vehicle control at study termination. In addition the mean platelet count of the high dose males was significantly elevated compared to vehicle control at study termination. The low and high dose males and females exhibited slight (low dose) to statistically significant (high dose) increases in mean cholesterol levels at termination. In addition statistically significant decreases were observed in the mean albumin levels of the high dose males and females. Total protein and albumin/globulin ratios were normal. The mean plasma, erythrocyte and brain cholinesterase values of the low and high dose females and high dose males were reduced compared to the vehicle control. Differences from vehicle control were for the most part statistically significant and ranged from –12% to –39%.</p>

	<p>The differences cited from vehicle control in hematology, clinical chemistry and cholinesterase parameters were considered treatment related.</p> <p>The mean and relative (to body weight) testes and epididymidal weights were markedly lower than vehicle control in the high dose group. The mean absolute testes weight was reduced 47% and the mean absolute epididymidal weight was reduced 43% compared to vehicle control. Testes and epididymidal weights in the low dose group were unremarkable. The mean absolute and relative (to body weight) adrenal weights of the high dose males and females were slightly to statistically significantly elevated compared to vehicle control. The mean absolute adrenal weight was increased 23% in the males and 46% in the females. The mean relative adrenal weights were increased 48% in the males and 80% in the females compared to vehicle control. Absolute and relative adrenal weights in the low dose males and females were considered comparable to vehicle control. Slight to statistically significant, dose related increases in mean absolute and relative kidney weights were evident in the low and high dose males and females.</p> <p>Macroscopic examinations conducted at study termination confirmed the in life dermal observations of the treated animals. Macroscopic dermal changes included atonia, alopecia, exfoliation, fissuring and eschar formation. In addition the testes of the high dose animals were observed to be markedly smaller than those of the vehicle control males.</p> <p>Compound related microscopic changes observed in the low and high dose males and females consisted of slight to moderately severe hyperkeratosis, parakeratosis and epithelial hyperplasia. In some rabbits, these changes were accompanied by an increase in the amount of collagen present in the dermis and/or focal to multifocal areas of suppurative dermatitis. The degree of severity of these lesions was greater at the high dose level. There was no demonstrable difference between the skin changes of the male and female rabbits.</p> <p>Microscopic examination of the testes from both dose groups determined the presence of morphologic abnormalities in the seminiferous tubules of the testes of the high dose animals only that were characterized by aspermatogenesis, diffuse tubular hypoplasia and a reduced mitotic activity. These findings were considered treatment related. No treatment related microscopic findings were observed in the adrenal glands of the high dose males or females.</p> <p>A number of the vehicle control and treated animals exhibited congestion and edema of the lungs. Subchronic inflammatory changes consisting of multifocal pneumonitis were present in some vehicle control and treated animals. None of the pulmonary findings were attributed to the topical administration of the test material.</p>
<u>Conclusions</u>	Based on the findings observed during this study, at the low dose level, this reviewer has concluded that an NOAEL was not established for this study.
<u>Data Quality</u>	Reliable without restriction (Klimisch Code)
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 12/30/01

Robust Summary 5-Repeated Tox-5

<u>Test Substance</u>	
CAS #	28629-66-5
Chemical Name	isooctyl derivative
Remarks	Test material purity not provided.
<u>Method</u>	
Method/Guideline followed	OECD 410
Test Type	28-day dermal toxicity study in rabbits
GLP (Y/N)	Y
Year (Study Performed)	1980
Species	Rabbit
Strain	New Zealand White (SPF)
Route of administration	Dermal, 5 days/week, to the clipped, unabraded, dorsal surface.
Duration of test	20 days of treatment
Doses/concentration levels	0, 5 and 25% (w/v) (OECD Guideline 410 suggests three treated groups and a control be included in this study design. The lowest dose level should be free of toxic effects. These suggestions were not met in this study. This was a two-treated group study. Effects were seen at the lowest dose level.)
Vehicle control	Yes (Primol 185)
Dose volume	2 mL/kg/day
Analytical confirmation of dose concentration	Yes
Sex	Males and females
Frequency of treatment	Once/day, 5 days/week for a total of 20 doses.
Vehicle control and treatment groups	Ten male and ten female rabbits in the vehicle control group and in both treated groups. An untreated control group was not included in the study.
Post exposure observation period	None
Statistical methods	Body weight, hematology and clinical chemistry parameters, organ weights and organ/body weight ratios were analyzed. Mean values of all dose groups were compared to control at each time interval. Tests included parametric ANOVA with a Dunnett's test and regression analysis for linear response, non-parametric Kruskal-Wallis and Dunn's Summed Rank Test, Jonckheere's test for monotonic trend.
Remarks field for test conditions	The test material was applied to the clipped, unabraded dorsal surface of the rabbits for, 5 days/week for 20 days. Elizabethan collars were used to prevent ingestion. (OECD Guideline 410 suggests the use of a gauze patch over the treatment site secured to the trunk with non-irritating tape and wrapped with an elastic sleeve. This procedure was not used during this study. This is considered a minor deviation from the Guideline.) The backs of all animals were gently wiped with paper towels approximately 6 hours after exposure to remove excessive test substance, if necessary. Mortality and gross signs of toxicological and pharmacological effects were evaluated twice daily. Clinical examinations were made weekly. Dermal responses were evaluated daily (7 days/week). Body weight was recorded weekly during treatment. Hematology and clinical chemistry parameters were evaluated pretest and at termination of treatment. Macroscopic examinations were performed on all animals. Select organs were weighed. Select tissues were examined microscopically for all

	animals in the vehicle control and high dose group. The testes and gross changes and tissue masses were evaluated for all low dose animals.
<u>Results</u>	
Remarks	<p>One high dose male and three high dose females died prior to study termination. These animals were found dead on test days 17, 23, 29 and 30. The cause of these deaths was not determined. All of the remaining vehicle control and treated animals survived the four-week duration of the study.</p> <p>Emaciation was a common finding in the 5% and 25% treated males and females. The incidence and severity of this finding were dose related. The high dose females were most affected. Dermal thickening was observed in most of the treated animals during the last two weeks of study. Ano-genital staining, nasal discharge and lacrimation were frequently observed in the treated males and females at both dose levels. Lacrimation was observed in 2 vehicle control males and in 6 vehicle control females during the last week of the study.</p> <p>Low and high dose males and females generally exhibited marked, dose related increases in the incidence and severity of erythema, edema, atonia desquamation, fissuring, eschar formation and exfoliation. Many of these observations were moderate to extreme in severity, particularly those in the high dose group. Dermal irritation was observed in the vehicle control group. Severity and incidence were lower in the vehicle controls than in the test material treated animals.</p> <p>The mean body weights of the low and high dose males and females were reduced compared to vehicle control throughout the study. Differences from vehicle control in the low and high dose males ranged from 3 to 17%, and in the low and high dose females ranged from 10 to 23%. These differences from vehicle control were considered treatment related.</p> <p>Mean hemoglobin, hematocrit and erythrocyte counts were slightly to statistically significantly reduced compared to vehicle control at study termination. Slight to statistically significant differences from vehicle control were observed in several clinical chemistry parameters of the test material treated animals. These included: decreased alkaline phosphatase (25% females), decreased albumin (25% males/females), increased blood urea nitrogen (25% males/females), increased cholesterol (5 and 25% males/females) and increased total and direct bilirubin (5 and 25% females). In addition mean brain cholinesterase levels were slightly reduced (-14 to -17%) in the 5 and 25% males and females. These differences from vehicle control in hematology and clinical chemistry parameters were considered treatment related.</p> <p>The mean and relative (to body weight) testes and epididymides weights were markedly lower than control in the low and high dose groups. Differences from control were dose related and were considered treatment related.</p> <p>Macroscopic and microscopic examinations conducted at study termination confirmed the in life dermal observations of the treated animals. Microscopic dermal changes consisted of slight to moderately severe hyperkeratosis,</p>

	<p>parakeratosis and epithelial hyperplasia. In some rabbits, these changes were accompanied by an increase in the amount of collagen present in the dermis and/or focal to multifocal areas of suppurative dermatitis. The degree of severity of these lesions was greater at the high dose level. There was no demonstrable difference between the skin changes of the male and female rabbits. At necropsy the testes of the 5 and 25% dose group animals were markedly smaller than those of the vehicle controls. Microscopic examination of the testes from both dose groups determined the presence of morphologic abnormalities in the seminiferous tubules of the testes that were characterized by aspermatogenesis, diffuse tubular hypoplasia and a reduced mitotic activity. These findings were considered treatment related.</p>
<u>Conclusions</u>	Based on the findings observed during this study, at the low dose level, this reviewer has concluded that an NOAEL was not established for this study.
<u>Data Quality</u>	Reliable without restriction (Klimisch Code)
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 9/13/01

Robust Summary 5-Repeated Tox-6

<u>Test Substance</u>	
CAS #	CAS# 2215-35-2
Chemical Name	2-pentanol, 4-methyl-, hydrogen phosphorodithioate, Zn salt
Remarks	Test material purity not provided.
<u>Method</u>	
Method/Guideline followed	43 CFR 163.82-2
Test Type	3 week dermal toxicity study in rabbits
GLP (Y/N)	N
Year (Study Performed)	1979
Species	Rabbit
Strain	Albino
Route of administration	Dermal, 5 days/week, to the clipped, dorsal surface.
Duration of test	Three weeks, 15 days of treatment
Doses/concentration levels	0, 0.8 and 1.6 ml/kg
Vehicle control	None
Sex	Males/Females
Frequency of treatment	Once/day, 5 days/week for a total of 15 doses.
Control and treatment groups	3/sex/group unabraded 3/sex/group abraded (Due to an apparent incorrect sexing 2 abraded control males and 4 abraded control females were placed on study.)
Post exposure observation period	None
Statistical methods	Analysis of variance, Newman-Keuls analysis
Remarks field for test conditions	The test material was applied to the clipped, unabraded or abraded dorsal surface of the rabbits 5 days/week for 3 weeks. Abraded animals were abraded once per week. The treated skin was covered with gauze patches secured with hypoallergenic tape, and covered with an impervious wrap that was held in place with an elastic bandage. Treatment sites were occluded for 6 hours/day, 5 days/week. Control animals were handled in an identical fashion as the treated animals, however, they were untreated. Clinical observations were made daily. Dermal responses were evaluated daily, on treatment days, immediately prior to the next application of test material (Draize). Body weights and food consumption were determined every 3 to 4 days. Hematology and clinical chemistry parameters were evaluated pretest and at termination of treatment. Macroscopic examinations were performed on all animals. Select tissues were weighed. A range of tissues was examined microscopically for all animals.
<u>Results</u>	
Remarks	A total of three males and three females were found dead or were sacrificed moribund during the study. Two high dose males (Group II, unabraded) were sacrificed moribund on test days 16 and 19 respectfully. The cause of death of these animals was not determined. One high dose female (Group II, unabraded) died during terminal bleeding. The cause of death was attributed to pneumonia. One low dose female (Group I, unabraded) died on test day 21. The pathologist attributed this animal's death to infection and pneumonia. One Control female (Group III, abraded) died on test day 22 following bleeding. Death was

attributed to hemorrhage following intracardiac bleeding. One Control male (Group III, abraded) was found dead on test day 15. This animal's death was probably due to the presence of severe pneumonitis.

Dermal irritation (slight to moderate erythema and edema) was present on test day 1 in both abraded and unabraded low and high dose male and female animals. By test days 3 - 4 severe erythema and severe edema were present in the abraded and unabraded males and females at both dose levels. Skin cracking was observed in all treated animals by the end of the first week of study. The severity of findings was essentially the same in the low and high dose animals. Differences in findings by dose level were not apparent.

All of the abraded and unabraded high dose males and females exhibited a body weight loss over the 21-day study period. The low dose unabraded female that died during the study exhibited a progressive weight loss throughout the study up until the time it died on test day 21. Two of three unabraded low dose males and one of the two surviving low dose unabraded females lost weight during the treatment period. All of the abraded low dose males and 2 of 3 abraded low dose females gained weight during the study. The remaining abraded low dose female lost weight during the last ten days of study. In general the mean food consumption values of the surviving low and high dose animals were lower than control, suggesting a dose related effect of test material administration.

The mean total white blood cell count was elevated in the males in the low (unabraded only) and high (abraded and unabraded) dose groups. The increases observed in the high dose males were attributed to treatment. Observed alterations in clinical chemistry parameters included: triglyceride- increased in the high dose, abraded and unabraded, males and females; uric acid- increased in the high dose, abraded and unabraded, females; SGOT- increased in the high dose abraded females, only and LDH- increased in the high dose, abraded and unabraded, females. These increases, in the high dose group only, suggest a relationship to treatment with the test material. The mean GGT level of the high dose abraded females was also elevated compared to control. This increased value was attributed to an increase in one of the three abraded high dose animals only. This increase was not considered treatment related.

The mean testes weights, testes/body weight ratios and testes to brain weight ratios of the high dose, abraded and unabraded, males were lower than control. These differences from control were considered treatment related. The liver and heart weights and the liver and heart to brain weight ratios of the high dose males (abraded and unabraded) were reduced compared to controls. The differences from control in heart and liver weights were not associated with any microscopic findings in these organs. For this reason, these differences from control (heart and liver) were not attributed to treatment. The mean ovary weights, ovary/body weight ratios and ovary to brain weight ratios of the high dose, abraded and unabraded, females were lower than control. The differences from control in absolute and relative ovary weights were not associated with any microscopic findings. For this reason, these differences from control were not attributed to treatment.

	<p>Skin necrosis with epithelial hyperplasia upon re-epithelialization, subacute interstitial pneumonia and depletion of lymphoid organs due to stress and weight loss were observed microscopically in both the low and high dose groups. The findings were more severe in the high dose. No significant microscopic pathology was observed in the heart, liver, gastrointestinal tract, kidneys, urinary bladder, adrenals, thyroid or brain.</p> <p>Of the six male animals in the high dose, the testes of the three rabbits with unabraded skin showed decreased spermatogenesis, the testes of one rabbit with abraded skin showed increased multinucleate forms. The testes of the three male high dose animals with abraded skin exhibited no pathologic changes. No inflammatory changes were seen in the testes of the animals which showed reduced or altered spermatogenesis. Similar changes were not seen in either the control or low dose group except for two low dose males that had slightly reduced spermatogenic activity. The decreased spermatogenic activity observed in the high dose group was attributed to exposure to the test material.</p>
<u>Conclusions</u>	Based on the findings observed during this study this reviewer has concluded that an NOAEL was not established for this study. Significant toxicity was observed at both concentrations of the test material tested
<u>Data Quality</u>	Reliable with restriction (Klimisch Code). Restriction due to the fact that this study predates the EPA GLPs and that, physical observation data, other than irritation findings, were not reported. In addition many of the animals had evidence of respiratory disease that was common in rabbit studies at the time that this study was conducted.
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 12/10/01

2.3 Genetic Toxicity

Robust Summary 5-GenTox-1

<u>Test Substance</u>	
CAS #	CAS# 26566-95-0
Chemical Name	Phosphorodithioic acid, mixed 0-(2-ethylhexyl)- O-(2-methylpropyl) esters, zinc salts
Remarks	Test material purity not provided.
<u>Method</u>	
Method/Guideline followed	Schechtman, L., Kouri, R., Progress in Genetic Toxicology, Elsevier/North-Holland Biomedical Press, New York, pp. 307-316 (1977).
Test Type	In vitro point mutation assay using BALB/3T3 mouse embryo cells without metabolic activation
GLP (Y/N)	Y
Year (Study Performed)	1981
Test System	BALB/3T3 mouse embryo cells
Exposure Method	Dilution
Test Substance Doses/concentration levels	Concentrations of 30, 10 and 3 ug/mL were evaluated without metabolic activation.
Metabolic Activation	None
Vehicle	The test material was solubilized in acetone and diluted to the appropriate concentration in aqueous cell culture medium. Solvent control plates were treated with acetone at the same concentration needed to expose the target cells to the highest dose of test article in complete medium.
Positive Control concentration level	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG): 0.5 ug/ml
Statistical Analysis	The transforming potential of each treatment condition was compared to that of the solvent control using the Poisson distribution.
Test Substance Solubility	Test substance was solubilized in acetone.
Dose rangefinding study	Yes
Remarks field for test conditions	Exponentially growing 3T3 clone A31-1 cells were seeded for an evaluation of cytotoxicity at 250 cells/culture in triplicate/condition, and at 1×10^4 cells/plate in 12-15 replicates for the mutation assay. Plates were incubated at 36° C in a humidified atmosphere of 5% CO ₂ in air for 20-24 hours. Cells were exposed to three concentrations of test article as well as solvent and positive controls for 24 hours. Cells were then washed and the treated media replaced with untreated growth medium. After 7-10 days incubation, the concurrent cytotoxicity plates were fixed with methanol, stained with 10% Giemsa, and scored for colony formation. After the 4-6 day expression period necessary for fixation of the genotypic lesion which evolves as the mutant phenotype, the number of cells in a representative mutation assay plate for each test condition was determined in order to establish the number of cells at risk to the selective agent (ouabain). Cells in the remaining mutation assay plates were then treated with complete medium containing 1mM ouabain. Cells were incubated at 36° C with scheduled medium/ouabain changes for 4-6 weeks. Cells were then fixed, stained and scored for the development of ouabain-resistant colonies.

	<p>The cytotoxic effects of each treatment condition were expressed relative to the solvent treated control (relative cloning efficiency). The number of colonies forming/number of cells seeded/treatment condition were determined for the positive and negative controls and the treated groups. The CAR (number of cells at risk to ouabain) for each treatment group was calculated from the cell counts of representative mutation assay plates for each group. The mutation results were expressed in terms of mutation frequencies (the number of ouabain resistant mutant colonies/number of CAR/condition).</p> <p>For a valid test the cloning efficiency of the solvent control must be greater than or equal to 15%. The relative survival of cells exposed to the test article must be equal to or greater than 40% for at least one dose level. The number of spontaneous ouabain-resistant mutants in the negative control must not exceed 4/total replicate plates. The positive control must induce a significant ($p \leq 0.05$) number of ouabain-resistant mutants relative to the negative control.</p>
<u>Results</u>	The test substance exhibited positive mutagenic activity in 3T3 cells in the absence of metabolic activation.
Remarks	Relative to solvent control cell survival was 85%, 67% and 0.3% at 3, 10 and 30 ug/ml respectively. The positive control reduced the colony forming efficiency of the 3T3 cells by 97%. Three spontaneous ouabain-resistant colonies were observed in the vehicle control. The test material induced 132 ouabain-resistant colonies at 10 ug/ml and 16 at 3 ug/ml. Mutation frequencies in these groups and in the positive control were statistically significant compared to the vehicle control.
<u>Conclusions</u>	The test substance exhibited positive mutagenic activity in 3T3 cells in the absence of metabolic activation.
<u>Data Quality</u>	Reliable without restriction (Klimisch Code)
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 8/7/00 (RTA-067)

Robust Summary 5-GenTox-2

<u>Test Substance</u>	
CAS #	CAS# 26566-95-0
Chemical Name	Phosphorodithioic acid, mixed 0-(2-ethylhexyl)- O-(2-methylpropyl) esters, zinc salts
Remarks	Test material purity not provided.
<u>Method</u>	
Method/Guideline followed	Schechtman, L., Kouri, R., Progress in Genetic Toxicology, Elsevier/North-Holland Biomedical Press, New York, pp. 307-316 (1977).
Test Type	In vitro point mutation assay using BALB/3T3 mouse embryo cells with metabolic activation
GLP (Y/N)	Y
Year (Study Performed)	1981
Test System	BALB/3T3 mouse embryo cells
Exposure Method	Dilution
Test Substance Doses/concentration levels	Concentrations of 3, 1 and 0.3 ug/mL were evaluated with metabolic activation.
Metabolic Activation	Yes
Vehicle	The test material was solubilized in acetone and diluted to the appropriate concentration in aqueous cell culture medium. Solvent control plates were treated with acetone at the same concentration needed to expose the target cells to the highest dose of test article in complete medium.
Positive Control concentration level	Benzo(a)pyrene, 12.5 ug/ml
Statistical Analysis	The transforming potential of each treatment condition was compared to that of the solvent control using the Poisson distribution.
Test Substance Solubility	Test substance was solubilized in acetone.
Dose rangefinding study	Yes
Remarks field for test conditions	<p>Exponentially growing 3T3 clone A31 cells were seeded for the evaluation of cytotoxicity and mutagenicity at 250 cells/culture in triplicate/condition. Cells were treated in suspension in a reaction mixture containing the cofactor pool (NADPH), S-9 and test article or control compounds and were incubated at 36° C in a humidified atmosphere of 5% CO₂ in air for 2 hours. After the exposure period, the target cells were washed with HBSS and resuspended in complete medium.</p> <p>For the cytotoxicity assay, cells were seeded in complete medium at 250 cells/plate in triplicate/condition for the determination of the relative cytotoxic effects of each treatment. Cells were incubated at 36° C with scheduled medium changes for approximately 7 to 10 days. The cytotoxicity plates were fixed with methanol, stained with 10% Giemsa, and scored for colony formation in order to determine the relative plating efficiency per condition.</p> <p>For the mutation assay, the remaining cells from each test condition were cultured further in complete medium at 36° C for the 4-6 day expression period necessary for fixation of the genotypic lesion, which evolves as the mutant phenotype. The cells were then collected and an aliquot from each condition was seeded at 250 cells/plate in triplicate/condition and incubated at 36° C with scheduled medium changes. After approximately 7 to 10 days, the plates were</p>

	<p>fixed with methanol, stained with 10% Giemsa, and scored for colony formation in order to determine the relative plating efficiency per condition and in order to establish the number of cells at risk to the selective agent (ouabain). Cells in the remaining mutation assay plates were then treated with complete medium containing 1mM ouabain.</p> <p>Induction of mutation was determined by seeding approximately 2×10^5 cells/dish, in complete medium, from those remaining cells collected post expression, into 12-15 dishes/condition. Plates were incubated for 4 hours. Ouabain (5mM) was added to each culture to a final concentration of 1 mM in complete medium. Cells were incubated with ouabain/medium changes for 4-6 weeks. Cells were then fixed, stained and scored for the development of ouabain-resistant colonies.</p> <p>The cytotoxic effects of each treatment condition were expressed relative to the solvent treated control (relative cloning efficiency). The number of colonies forming/number of cells seeded/treatment condition were determined for the positive and negative controls and the treated groups. The CAR (number of cells at risk to ouabain) for each treatment group was calculated from the cell counts of representative mutation assay plates for each group. The mutation results were expressed in terms of mutation frequencies (the number of ouabain resistant mutant colonies/number of CAR/condition).</p> <p>For a valid test the cloning efficiency of the solvent control must be greater than or equal to 15%. The relative survival of cells exposed to the test article must be equal to or greater than 40% for at least one dose level. The number of spontaneous ouabain-resistant mutants in the negative control must not exceed 4/total replicate plates. The positive control must induce a significant ($p \leq 0.05$) number of ouabain-resistant mutants relative to the negative control.</p>
<u>Results</u>	The test substance exhibited positive mutagenic activity in 3T3 cells in the presence of metabolic activation.
Remarks	Relative to solvent control cell survival was 94%, 81% and 80% at 0.3, 1.0 and 3.0 ug/ml respectively. The positive control reduced the colony forming efficiency of the 3T3 cells by 26%. Four spontaneous ouabain-resistant colonies were observed in the vehicle control. The test material induced 11 ouabain-resistant colonies at 3 ug/ml, 8 at 1 ug/ml and 21 at 0.3 ug/ml. Mutation frequencies in these groups and in the positive control were statistically significant when compared to the vehicle control.
<u>Conclusions</u>	The test substance exhibited positive mutagenic activity in 3T3 cells in the presence of metabolic activation.
<u>Data Quality</u>	Reliable without restriction (Klimisch Code)
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 2/23/01 (RTA-075)

Robust Summary 5-GenTox-3

<u>Test Substance</u>	
CAS #	CAS# 26566-95-0
Chemical Name	Phosphorodithioic acid, mixed 0-(2-ethylhexyl)- O-(2-methylpropyl) esters, zinc salts
Remarks	Test material purity not provided.
<u>Method</u>	
Method/Guideline followed	Mutation Research 31: 9-15 (1975)
Test Type	Mammalian Erythrocyte Micronucleus Test
GLP (Y/N)	Y
Year (Study Performed)	1980
Species	Mouse
Strain	Swiss Albino CrI:CD-1
Route of administration	Intraperitoneal
Duration of test	Two doses 24 hours apart followed by a 6-hour evaluation period.
Doses/concentration levels	0, 10 and 20 mg/kg
Dose volume	20 ml/kg
Sex	Males and females
Frequency of treatment	Twice (at initiation and at 24 hours)
Control and treatment groups	0.25% Methylcellulose and Tween 80 vehicle control: 4/sex; triethylenemelamine positive control: 0.5 mg/kg, 4/sex; 10 and 20 mg/kg: 4/sex
Statistical methods	Statistically significant differences were evaluated in the frequency of micronucleated polychromatic erythrocytes between treated groups and vehicle controls using a Student's t test.
Dose rangefinding study	10, 30, 100, 300 and 6000 mg/kg Mortality and physical observations were evaluated.
Remarks field for test conditions	All animals observed frequently for physiological or behavioral abnormalities on the day of dosing and periodically thereafter. Body weights taken on first day of the study prior to treatment. All animals from each treatment group and vehicle control group were sacrificed for bone marrow sampling 6 hours after the last treatment. The frequency of micronucleated cells was expressed as percent micronucleated cells versus total polychromatic erythrocytes. (This study design deviates significantly from OECD Guideline 474. Differences include the number of administrations of test material (2x vs 1x), number of animals/sex (4 vs 5), number of dose levels evaluated (2 vs 3), and number of evaluation time points (1 vs 3).)
<u>Results</u>	
Remarks	<p>During the dose rangefinding study significant mortality was observed at all levels above 30 mg/kg. The 10 and 30 mg/kg dose levels exhibited hypoactivity and writhing after dose administration. Based on these data dose levels of 10 and 20 mg/kg were selected for the main study.</p> <p>During the main study test material treated animals exhibited hypoactivity and piloerection post dosing. No deaths occurred. No statistically significant increases in micronucleated polychromatic</p>

	erythrocytes over the levels observed in the vehicle controls were observed in either sex. The variability in response observed in the treated animals was similar to that observed in the vehicle control. The positive control exhibited a statistically significant increase in micronuclei as expected.
<u>Conclusions</u>	Under the conditions of this study the test material did not induce micronuclei in bone marrow erythrocytes of mice.
<u>Data Quality</u>	Reliable with restriction (Klimisch Code). Restriction based on the number and type of deviations in the study design from OECD Guideline 474.
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 7/18/00 (RTA-060)

Robust Summary 5-GenTox-4

<u>Test Substance</u>				
CAS #	CAS# 26566-95-0			
Chemical Name	Phosphorodithioic acid, mixed 0-(2-ethylhexyl)- O-(2-methylpropyl) esters, zinc salts			
Remarks	Test material purity not provided.			
<u>Method</u>				
Method/Guideline followed	OECD Guideline 471			
Test Type	Bacterial Reverse Mutation Assay			
GLP (Y/N)	N			
Year (Study Performed)	1981			
Test System	<i>Salmonella typhimurium</i>			
Strains Tested	<i>Salmonella typhimurium</i> tester strains TA98, TA100, TA1535, TA1537, TA1538			
Exposure Method	Plate incorporation			
Test Substance Doses/concentration levels	0.1, 0.03, 0.01, 0.003 and 0.001 ul/plate without activation 0.3, 0.1, 0.03, 0.01 and 0.003 ul/plate with activation			
Metabolic Activation	With and without (S9 fraction mix of livers of Aroclor 1254 pretreated Sprague Dawley rats)			
Vehicle	Ethanol			
Tester strain, activation status, Positive Controls and concentration level	TA98	+S9	2-aminoanthracene	5 ug/plate
	TA98	-S9	2-nitrofluorene	5 ug/plate
	TA100	+S9	2-aminoanthracene	5 ug/plate
	TA100	-S9	sodium azide	30 ug/plate
	TA1535	+S9	2-aminoanthracene	5 ug/plate
	TA1535	-S9	sodium azide	30 ug/plate
	TA1537	+S9	2-aminoanthracene	5 ug/plate
	TA1537	-S9	9-aminoacridine	10 ug/plate
	TA1538	+S9	2-aminoanthracene	5 ug/plate
	TA1538	-S9	2-nitrofluorene	5 ug/plate
Vehicle Control	Acetone			
Statistical Analysis	Mean revertant colony count and standard deviation were determined for each dose point.			
Dose Rangefinding Study	No			
S9 Optimization Study	No			
Remarks field for test conditions	<p>This study was conducted prior to the development of OECD Guideline No. 471. This study deviates from the guideline in that Tester Strain TA 1538 is not called for in the guideline but it was included. In addition E. coli WP2 urvA Tester Strain called for in the guideline was not include.</p> <p>There were two treatment sets for each tester strain, with (+S9) and without (-S9) metabolic activation. Each of the tester strains was dosed with five concentrations of test substance, vehicle controls, and a positive control. Three plates/dose group/strain/treatment set were evaluated. 100 ul of test material, positive control or vehicle control were added to each plate along with 100 ul of tester strain, S9 mix (if</p>			

	needed) and 2.0 ml of top agar. This was overlaid onto the surface of minimal bottom agar in a petri dish. Plates were incubated for 48 hours at 37°C.
<u>Results</u>	The test substance was not genotoxic in this assay with or without metabolic activation.
Remarks	<p>All data were acceptable and no positive increases in the number of revertants/plate were observed with any of the tester strains with or without metabolic activation.</p> <p>The positive control for each respective test strain exhibited at least a 5-fold increase (with or without S9) over the mean value of the vehicle control for a given strain, confirming the expected positive control response.</p>
<u>Conclusions</u>	Under the conditions of this study, the test material was not mutagenic.
<u>Data Quality</u>	Reliable with restriction (Klimisch Code). Restriction due to the lack of any information regarding the selection of dose levels used during the study. In addition no information is presented regarding cytotoxicity or the presence of test material precipitate in the cultures.
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 7/16/00 (RTA-056)

Robust Summary 5-GenTox-5

<u>Test Substance</u>	
CAS #	CAS# 4259-15-8
Chemical Name	2-ethyl hexyl derivative
Remarks	Test material purity not provided.
<u>Method</u>	
Method/Guideline followed	OECD Guideline 474
Test Type	Mammalian Erythrocyte Micronucleus Test
GLP (Y/N)	Y
Year (Study Performed)	1996
Species	Mouse
Strain	Swiss Albino CrI:CD-1 (ICR)BR
Route of administration	Intraperitoneal
Duration of test	Single dose followed by 72-hour evaluation period.
Doses/concentration levels	0, 6, 12 and 24 mg/kg
Dose volume	10 ml/kg
Sex	Males and females
Frequency of treatment	Once
Control and treatment groups	Peanut oil vehicle control: 15/sex; cyclophosphamide positive control: 60 mg/kg, 5/sex; 6 and 12 mg/kg: 15/sex; 24 mg/kg: 20/sex
Statistical methods	Animal to animal variability in spontaneous frequency of micronucleated polychromatic erythrocytes was evaluated in vehicle controls. Statistically significant differences were evaluated in the frequency of micronucleated polychromatic erythrocytes between treated groups and vehicle controls. NCE/PCE (normochromatic erythrocytes/polychromatic erythrocytes) ratios in treated and control groups were compared. Tests included Cochran-Armitage test for trend, a one-way analysis of variance and Dunnett's procedure.
Dose rangefinding study	Study I: 550, 1787, 3024, 4261 and 5498 mg/kg Study II: 25, 37.5, 50, 75 and 100 mg/kg Mortality and physical observations were evaluated.
Remarks field for test conditions	All animals observed frequently for physiological or behavioral abnormalities on the day of dosing and periodically thereafter. Body weights taken on first day of the study prior to treatment. Five/sex from each treatment group and vehicle control group were sacrificed for bone marrow sampling 24, 48 and 72 hours post treatment. Positive controls sampled at 24 hours only. NCE/PCE ratio and %PCE of total erythrocytes were calculated by counting a total of ≥ 1000 erythrocytes/animal. A total of 1000 PCE /animal were evaluated for the presence of micronuclei. (Guideline calls for 2000/animal to be evaluated. This difference from the current guideline was not considered sufficient to effect the reliability of the study.)
<u>Results</u>	
Remarks	During the first dose rangefinding study significant mortality was observed at all levels. Hypoactivity and signs of moribundity were observed in most animals at all dose levels. During the second dose

	<p>range-finding study significant mortality was observed at all levels. At the low dose (25 mg/kg) 1 male and all 3 females survived. Hypoactivity, tremors and/or squinting eyes were noted in a number of the treated animals. Based on these data dose levels of 0, 6.0, 12 and 24 mg/kg were selected for the main study.</p> <p>During the main study one 72-hour low dose female died shortly after dosing. Upon necropsy this death was attributed to a dosing accident. This animal was replaced. Between 20 hours post dosing and study termination 7 male and 2 female high dose animals were found dead. Several of these animals exhibited hypoactivity, tremors and were prostrate prior to death. Slight hypoactivity was observed at 12 mg/kg and higher on the day of dosing.</p> <p>No statistically significant increases in micronucleated polychromatic erythrocytes over the levels observed in the vehicle controls were observed in either sex or at any harvest time point. All values for individual animals were within the expected range of micronucleated-PCE/1000 PCE expected for control animals. The variability in response observed in the treated animals was similar to that observed in the vehicle control. The positive control exhibited a statistically significant increase in micronuclei as expected. The test article did induce a statistically significant decrease in the PCE:NCE ratio of the high dose females at 48 hours.</p>
<u>Conclusions</u>	Under the conditions of this study the test material did not induce micronuclei in bone marrow erythrocytes of mice.
<u>Data Quality</u>	Reliable without restriction (Klimisch Code)
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 7/18/00 (RTA-059)

Robust Summary 5-GenTox-6

<u>Test Substance</u>	
CAS #	CAS# 4259-15-8
Chemical Name	2-ethyl hexyl derivative
Remarks	Test material purity not provided.
<u>Method</u>	
Method/Guideline followed	OECD Guideline 471
Test Type	Bacterial Reverse Mutation Assay
GLP (Y/N)	Y
Year (Study Performed)	1996
Test System	<i>Salmonella typhimurium</i> and <i>Escherichia Coli</i>
Strains Tested	<i>Salmonella typhimurium</i> tester strains TA98, TA100, TA1535, TA1537; <i>Escherichia Coli</i> tester strain WP2uvrA
Exposure Method	Plate incorporation
Test Substance Doses/concentration levels	<u>Initial assay:</u> <i>Salmonella</i> + (S9): 25, 50, 100, 250, 1,000, and 5,000 ug/plate <i>Salmonella</i> - (S9): 1.0, 5.0, 10.0, 25.0, 100, and 500 ug/plate WP2uvrA + (S9): 50, 100, 250, 500, 2,000, and 10,000 ug/plate WP2uvrA - (S9): 50, 100, 250, 500, 2,000 and 10,000 ug/plate <u>Confirmatory assay:</u> <i>Salmonella</i> + (S9): 50, 100, 250, 500, 1,000, and 5,000 ug/plate <i>Salmonella</i> - (S9): 5.0, 10.0, 25.0, 50.0, 100 and 500 ug/plate WP2uvrA + (S9): 100, 250, 500, 2,000, 5,000, and 10,000 ug/plate WP2uvrA - (S9): 50, 100, 250, 500, 2,000 and 10,000 ug/plate
Metabolic Activation	With and without (500 ul of 10% S9 fraction mix of livers of Aroclor 1254 pretreated Sprague Dawley rats)
Vehicle	Ethanol
Tester strain, activation status, Positive Controls and concentration level	TA98 +S9 benzo(a)pyrene 2.5 ug/plate TA98 -S9 2-nitroflourene 1.0 ug/plate TA100 +S9 2-aminoanthracene 2.5 ug/plate TA100 -S9 sodium azide 2.0 ug/plate TA1535 +S9 2-aminoanthracene 2.5 ug/plate TA1535 -S9 sodium azide 2.0 ug/plate TA1537 +S9 2-aminoanthracene 2.5 ug/plate TA1537 -S9 ICR-191 2.0 ug/plate WP2uvrA +S9 2-aminoanthracene 25.0 ug/plate WP2uvrA -S9 4-nitroquinoline-N-oxide 1.0 ug/plate
Vehicle Control	Ethanol
Statistical Analysis	Mean revertant colony count and standard deviation were determined for each dose point.
Dose Rangefinding Study	Conducted using tester strains TA100 and WP2uvrA and ten doses of test material ranging from 10.0 to 10,000 ug/plate, one plate/dose with (10% S9 homogenate/ml of S9 mix) and without metabolic activation. Cytotoxicity was evaluated.
S9 Optimization Study	Conducted using tester strains TA98 and TA100, and a cytotoxic dose level of test article (66.7 ug/plate) and four concentrations of S9 mix (5, 10, 20 and 80% S9 homogenate/ml of S9 mix). Cytotoxicity was

	evaluated.
Remarks field for test conditions	In the main study there were two treatment sets for each tester strain, with (+S9) and without (-S9) metabolic activation. Each of the tester strains was dosed with six concentrations of test substance, vehicle controls, and a positive control. Three plates/dose group/strain/treatment set were evaluated. The results of the initial assay were confirmed in a second independent experiment. 50 ul of test material, positive control or vehicle control were added to each plate along with 100 ul of tester strain, S9 mix (if needed) and 2.0 (with S9) or 2.5 ml (without S9) of top agar. This was overlaid onto the surface of 25 ml minimal bottom agar in a petri dish. Plates were incubated for 48 hours at 37°C. Plates that were not evaluated immediately were held at 5°C until evaluated. The condition of the bacterial background lawn was evaluated for cytotoxicity and test article precipitate.
<u>Results</u>	The test substance was not genotoxic in this assay with or without metabolic activation.
Remarks	<p>In the dose rangefinding study cytotoxicity was observed with tester strain TA100 at 333 ug/plate and above with metabolic activation and at 33.3 ug/plate and above without metabolic activation. With tester strain WP2uvrA cytotoxicity was observed at 667 ug/plate without activation and at 6,670 ug/plate with metabolic activation. Test article precipitate was observed on plates at 6,670 ug/plate and above with tester strain TA100 with and without activation. With WP2uvrA with metabolic activation precipitate was observed at 667 ug/plate and above. Without activation, with WP2uvrA, precipitate was observed at 3,330 ug/plate. Based on these results the dose levels outlined above (page 1, Test Substance Doses, Initial Assay) were selected.</p> <p>The S9 optimization study was performed using TA98 and TA100 with a non-cytotoxic dose of test article, (333 ug/plate) and four concentrations of S9 mix (5, 10, 20 and 80% S9 homogenate/ml of S9 mix). In the absence of any effect a 10% S9 mix was used in the mutagenicity study.</p> <p>In the initial and confirmatory mutagenicity assays all data were acceptable and no positive increases in the number of revertants/plate were observed with any of the tester strains with or without metabolic activation. Based on the results of the initial study the dilution factor between doses was reduced for the confirmatory study in the absence of metabolic activation. The doses outlined above (page 1, Test Substance Doses, Confirmatory Assay) were utilized.</p> <p>Cytotoxicity was observed at ≥ 50 ug/plate with the <i>Salmonella</i> tester strains with and without activation and at ≥ 250 ug/plate with WP2uvrA with and without activation. Test material participate was observed on plates at ≥ 500 ug/plate.</p> <p>The positive control for each respective test strain exhibited at least a 3-fold increase (with or without S9) over the mean value of the vehicle control for a given strain, confirming the expected positive control</p>

	response.
<u>Conclusions</u>	Under the conditions of this study, the test material was not mutagenic.
<u>Data Quality</u>	Reliable without restriction (Klimisch Code)
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 7/14/00 (RTA-054)

Robust Summary 5-GenTox-7

<u>Test Substance</u>	
CAS #	CAS# 68784-31-6
Chemical Name	Phosphorodithioic acid, mixed O,O-bis(sec-Bu and 1,3-dimethylbutyl) esters, zinc salts
Remarks	Test material purity not provided.
<u>Method</u>	
Method/Guideline followed	OECD Guideline 474
Test Type	Mammalian Erythrocyte Micronucleus Test
GLP (Y/N)	Y
Year (Study Performed)	1996
Species	Mouse
Strain	Swiss Albino Crl:CD-1 (ICR)BR
Route of administration	Intraperitoneal
Duration of test	Single dose followed by 72-hour evaluation period.
Doses/concentration levels	0, 12.5, 25 and 50 mg/kg
Dose volume	10 ml/kg
Sex	Males and females
Frequency of treatment	Once
Control and treatment groups	Peanut oil vehicle control: 15/sex; cyclophosphamide positive control: 60 mg/kg, 5/sex; 12.5 and 25 mg/kg: 15/sex; 50 mg/kg: 20/sex
Statistical methods	Animal to animal variability in spontaneous frequency of micronucleated polychromatic erythrocytes was evaluated in vehicle controls. Statistically significant differences were evaluated in the frequency of micronucleated polychromatic erythrocytes between treated groups and vehicle controls. NCE/PCE (normochromatic erythrocytes/polychromatic erythrocytes) ratios in treated and control groups were compared. Tests included Cochran-Armitage test for trend, a one-way analysis of variance and Dunnett's procedure.
Dose rangefinding study	12.5, 25, 50, 75 and 100 mg/kg Mortality and physical observations were evaluated.
Remarks field for test conditions	All animals observed frequently for physiological or behavioral abnormalities on the day of dosing and periodically thereafter. Body weights taken on first day of the study prior to treatment. Five/sex from each treatment group and vehicle control group were sacrificed for bone marrow sampling 24, 48 and 72 hours post treatment. Positive controls sampled at 24 hours only. NCE/PCE ratio and %PCE of total erythrocytes were calculated by counting a total of ≥ 1000 erythrocytes/animal. A total of 1000 PCE /animal were evaluated for the presence of micronuclei. (Guideline calls for 2000/animal to be evaluated. This difference from the current guideline was not considered sufficient to effect the reliability of the study.)
<u>Results</u>	
Remarks	During the dose rangefinding study significant mortality was observed at the 75 and 100 mg/kg dose levels. Hypoactivity was observed in

	<p>some animals at all dose levels during the first hour post dosing and at 50 mg/kg and higher at 18 hours post dosing. Based on these data dose levels of 0, 12.5, 25 and 50 mg/kg were selected for the study.</p> <p>During the main study hypoactivity was observed at 25 mg/kg and higher. At 50 mg/kg three males and two females were found dead between 17 and 67 hours post dosing.</p> <p>A statistically significant increase in micronucleated PCEs was observed in the high dose total for males and females at the 72-hour harvest time point. This increase was not attributed to test material exposure and was due to the low number of micronucleated PCEs in the concurrent control group. There were no significant increases for the high dose males or females at the 72-hour harvest individually, no observable dose response, and the value was within the historical range for the testing facility. No other significant increases in micronucleated PCEs over vehicle control levels occurred at any other harvest time.</p> <p>The positive control exhibited a statistically significant increase in micronuclei as expected. The test article did induce a statistically significant decrease in the PCE:NCE ratio of the high dose males at 48 hours.</p>
<u>Conclusions</u>	Under the conditions of this study the test material did not induce micronuclei in bone marrow erythrocytes of mice.
<u>Data Quality</u>	Reliable without restriction (Klimisch Code)
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 7/18/00 (RTA-058)

Robust Summary 5-GenTox-8

<u>Test Substance</u>	
CAS #	CAS# 68784-31-6
Chemical Name	Phosphorodithioic acid, mixed O,O-bis(sec-Bu and 1,3-dimethylbutyl) esters, zinc salts
Remarks	Test material purity not provided.
<u>Method</u>	
Method/Guideline followed	OECD Guideline 471
Test Type	Bacterial Reverse Mutation Assay
GLP (Y/N)	Y
Year (Study Performed)	1996
Test System	<i>Salmonella typhimurium</i> and <i>Escherichia Coli</i>
Strains Tested	<i>Salmonella typhimurium</i> tester strains TA98, TA100, TA1535, TA1537; <i>Escherichia Coli</i> tester strain WP2uvrA
Exposure Method	Plate incorporation
Test Substance Doses/concentration levels	<u>Initial assay:</u> <i>Salmonella</i> + (S9): 50, 100, 250, 500, 1,000, and 5,000 ug/plate <i>Salmonella</i> - (S9): 10, 25, 50, 100, 250 and 1,000 ug/plate WP2uvrA + (S9): 100, 250, 500, 1,000, 5,000, and 10,000 ug/plate WP2uvrA - (S9): 50, 100, 250, 500, 2,000 and 10,000 ug/plate <u>Confirmatory assay:</u> <i>Salmonella</i> + (S9): 50, 100, 250, 500, 1,000, and 5,000 ug/plate <i>Salmonella</i> - (S9): 50, 100, 250, 500, 1,000 and 5,000 ug/plate WP2uvrA + (S9): 100, 250, 500, 1,000, 5,000, and 10,000 ug/plate WP2uvrA - (S9): 100, 250, 500, 1,000, 5,000, and 10,000 ug/plate
Metabolic Activation	With and without (500 ul of 10% S9 fraction mix of livers of Aroclor 1254 pretreated Sprague Dawley rats)
Vehicle	Ethanol
Tester strain, activation status, Positive Controls and concentration level	TA98 +S9 benzo(a)pyrene 2.5 ug/plate TA98 -S9 2-nitrofluorene 1.0 ug/plate TA100 +S9 2-aminoanthracene 2.5 ug/plate TA100 -S9 sodium azide 2.0 ug/plate TA1535 +S9 2-aminoanthracene 2.5 ug/plate TA1535 -S9 sodium azide 2.0 ug/plate TA1537 +S9 2-aminoanthracene 2.5 ug/plate TA1537 -S9 ICR-191 2.0 ug/plate WP2uvrA +S9 2-aminoanthracene 25.0 ug/plate WP2uvrA -S9 4-nitroquinoline-N-oxide 1.0 ug/plate
Vehicle Control	Ethanol
Statistical Analysis	Mean revertant colony count and standard deviation were determined for each dose point.
Dose Rangefinding Study	Conducted using tester strains TA100 and WP2uvrA and ten doses of test material ranging from 10.0 to 10,000 ug/plate, one plate/dose with (10% S9 homogenate/ml of S9 mix) and without metabolic activation. Cytotoxicity was evaluated.
S9 Optimization Study	Conducted using tester strains TA98 and TA100, and a cytotoxic dose

	level of test article (333 ug/plate) and four concentrations of S9 mix (5, 10, 20 and 80% S9 homogenate/ml of S9 mix). Cytotoxicity was evaluated.
Remarks field for test conditions	In the main study there were two treatment sets for each tester strain, with (+S9) and without (-S9) metabolic activation. Each of the tester strains was dosed with six concentrations of test substance, vehicle controls, and a positive control. Three plates/dose group/strain/treatment set were evaluated. The results of the initial assay were confirmed in a second independent experiment. 50 ul of test material, positive control or vehicle control were added to each plate along with 100 ul of tester strain, S9 mix (if needed) and 2.0 (with S9) or 2.5 ml (without S9) of top agar. This was overlaid onto the surface of 25 ml minimal bottom agar in a petri dish. Plates were incubated for 48 hours at 37°C. Plates that were not evaluated immediately were held at 5°C until evaluated. The condition of the bacterial background lawn was evaluated for cytotoxicity and test article precipitate.
<u>Results</u>	The test substance was not genotoxic in this assay with or without metabolic activation.
Remarks	<p>In the dose rangefinding study cytotoxicity was observed with tester strain TA100 at 1,000 ug/plate and above with metabolic activation and at 333 ug/plate and above without metabolic activation. With tester strain WP2uvrA cytotoxicity was observed at 1,000 ug/plate without activation. Cytotoxicity was not observed with this tester strain with activation. Test article precipitate was observed on plates at 6,670 ug/plate and above with tester strain TA100 without activation. With TA100 with metabolic activation precipitate was observed on plates at 3,330 ug/plate and higher. With WP2uvrA with metabolic activation precipitate was observed at 667 ug/plate and above. Without activation, with WP2uvrA, precipitate was observed at 3,330 ug/plate. Based on these results the dose levels outlined above (page 1, Test Substance Doses, Initial Assay) were selected.</p> <p>The S9 optimization study was performed using TA98 and TA100 with a non-cytotoxic dose of test article, (333 ug/plate) and four concentrations of S9 mix (5, 10, 20 and 80% S9 homogenate/ml of S9 mix). In the absence of any effect a 10% S9 mix was used in the mutagenicity study.</p> <p>In the initial and confirmatory mutagenicity assay all data were acceptable and no positive increases in the number of revertants/plate were observed with any of the tester strains with or without metabolic activation. Based on the results of the initial study the dilution factor between doses was reduced for the confirmatory study in the absence of metabolic activation. The doses outlined above (page 1, Test Substance Doses, Confirmatory Assay) were utilized.</p> <p>Cytotoxicity was observed at $\geq 1,000$ ug/plate with the <i>Salmonella</i> and WP2uvrA tester strains with and without activation. Test material participate was observed on plates at $\geq 1,000$ ug/plate. In one trial</p>

	<p>precipitate was observed in the presence of S9 mix at a dose as low as 250 ug/plate.</p> <p>The positive control for each respective test strain exhibited at least a 3-fold increase (with or without S9) over the mean value of the vehicle control for a given strain, confirming the expected positive control response.</p>
<u>Conclusions</u>	Under the conditions of this study, the test material was not mutagenic.
<u>Data Quality</u>	Reliable without restriction (Klimisch Code)
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 7/14/00 (RTA-053)

Robust Summary 5-GenTox-9

<u>Test Substance</u>	
CAS #	CAS# 68988-46-5
Chemical Name	Phosphorodithioic acid mixed o,o-bis(iso-Bu, isooctyl, and pentyl) esters zinc salts
Remarks	Test material purity not provided.
<u>Method</u>	
Method/Guideline followed	OECD Guideline 476
Test Type	Mouse Lymphoma Mutagenicity Assay
GLP (Y/N)	Y
Year (Study Performed)	1983
Test System	L5178Y TK+/- mouse lymphoma cells
Exposure Method	Dilution
Test Substance Doses/concentration levels	Concentrations of 0.016, 0.012, 0.0089, 0.0067, 0.0050, 0.0038, 0.0028, 0.0021, 0.0016 and 0.0012 ul/mL were evaluated without metabolic activation. Concentrations of 0.032, 0.024, 0.018, 0.013, 0.010, 0.0075, 0.0056, 0.0042, 0.0032 and 0.0024 ul/mL were evaluated with metabolic activation.
Metabolic Activation	Aroclor induced rat liver
Vehicle	Acetone
Positive Control concentration levels by activation status	With activation: 7,12-dimethylbenzanthracene (DMBA) 5 and 7.5 ug/mL Without activation: ethylmethanesulfonate (EMS) 1 and 0.5 ul/mL
Statistical Analysis	Means and standard deviations were determined.
Test Substance Solubility	Test substance solubility in the vehicle was determined during a solubility test.
Dose rangefinding study	Test substance (dose levels from 0.001 to 10 ug/mL) and vehicle control tested with and without activation. Cultures were exposed to the test substance and incubated for approximately four hours, then washed and cultured for two days. Cell culture density was determined 24 and 48 hours post exposure. Treated cell suspension growth at each dose level was compared to the negative solvent control.
Remarks field for test conditions	<p>Prior to study initiation the solubility of the test substance and of the positive control materials in the vehicle was confirmed.</p> <p>In the main study there were two treatment sets for each concentration of test substance, with (+S9) and without (-S9) metabolic activation. DMBA (positive control) was tested with activation and EMS (positive control) was tested without activation. The test material was prepared so that the highest concentration was 100% toxic. The test material was added to cells with and without activation and incubated for four hours. Cells were then washed and placed in suspension cultures for two days with a cell population adjustment at 24 and 48 hours. The cells were then plated in a restrictive media containing trifluorothymidine (TFT) which allows TK^{-/-} cells to grow. Cells were also plated in a non-restrictive media that indicated cell viability. Plates were incubated at 37°C in a humidified 5% CO₂ atmosphere for 10-12 days. Following incubation all plates were scored for total number of colonies/plate. The frequency of mutation by dose was determined by comparing the average number of colonies in the mutagenicity plates to the average number of colonies</p>

	in the corresponding viability plates. For the study to be acceptable the following criteria must be met: mutation frequency of positive controls with or without activation should be twice that of the solvent control with a dose response.
<u>Results</u>	The test substance was not mutagenic in this assay without metabolic activation. The test substance was positive for mutagenic activity in the presence of metabolic activation.
Remarks	<p>The dose rangefinding study indicated a threshold level of complete toxicity at 0.05 ul/ml without activation and 0.1 ul/ml with activation. Based on these data the concentration ranges outlined above were selected for study in the main assay.</p> <p>In the main assay one culture cloned without activation exhibited a mutation frequency that was 4.8 times greater than the solvent control. This result was not considered significant since the total growth in this culture was 2%. None of the other cultures exhibited mutation frequencies that were significantly greater than control. The total growth of these cultures ranged from 12 to 109%. One metabolically activated culture (at the highest test material concentration tested) exhibited a mutation frequency that was significantly greater than the mutation frequency of the solvent controls. In addition, the cultures exhibited a dose response. Positive and vehicle control group responses were appropriate.</p>
<u>Conclusions</u>	The test substance was not mutagenic in this assay without metabolic activation. The test substance was positive for mutagenic activity in the presence of metabolic activation.
<u>Data Quality</u>	Reliable without restriction (Klimisch Code)
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 7/18/00 (RTA-061)

Robust Summary 5-GenTox-10

<u>Test Substance</u>	
CAS #	CAS# 68988-46-5
Chemical Name	Phosphorodithioic acid mixed o,o-bis(iso-Bu, isooctyl, and pentyl) esters zinc salts
Remarks	Test material purity not provided.
<u>Method</u>	
Method/Guideline followed	Schechtman, L., Kouri, R., Progress in Genetic Toxicology, Elsevier/North-Holland Biomedical Press, New York, pp. 307-316 (1977).
Test Type	Morphological transformation of BALB/3T3 mouse embryo cells without metabolic activation
GLP (Y/N)	Y
Year (Study Performed)	1983
Test System	BALB/3T3 mouse embryo cells
Exposure Method	Dilution
Test Substance Doses/concentration levels	Concentrations of 10, 20 and 30 ug/mL were evaluated without metabolic activation.
Metabolic Activation	None
Vehicle	The test material was solubilized in acetone and diluted to the appropriate concentration in Eagle's minimal essential medium (EMEM). Solvent control plates were treated with acetone at a final concentration of 2 ul/ml complete EMEM.
Positive Control concentration level	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG): 0.5 ug/ml
Statistical Analysis	The transforming potential of each treatment condition was compared to that of the solvent control using the Poisson distribution.
Test Substance Solubility	Test substance was solubilized in acetone.
Dose rangefinding study	Yes (clonal toxicity)
Remarks field for test conditions	<p>Exponentially growing 3T3 clone A31-1 cells were seeded for each treatment condition at 250 cells/ culture in 12-15 replicates for determination of phenotypic transformation and were incubated at 36° in a humidified atmosphere of 5% CO₂ in air for 20-24 hours. Cells were exposed to three concentrations of test article as well as solvent and positive controls for 24 hours. Cells were then washed and the treated media replaced with untreated growth medium. After 7-10 days incubation, the concurrent cytotoxicity plates were fixed with methanol, stained with 10% Giemsa, and scored for colony formation. After 4-6 weeks incubation with twice weekly medium changes, the transformation plates were fixed with methanol, stained with 10% Giemsa, and scored for morphologically transformed Type II and Type III foci.</p> <p>The cytotoxic effects of each treatment condition were expressed relative to the solvent treated control (relative cloning efficiency). The transformation frequency for each treatment group was expressed as the number of transformed foci/surviving cell. For a valid test the cloning efficiency of the solvent control must be greater than or equal to 25%. The relative survival of cells exposed to the test article must fall within 30-60% for one dose level and 60-90% for another dose level. The number of Type III foci in the negative control must not exceed one focus/total replicate plates. The positive control must</p>

	induce a significant ($p \leq 0.05$) number of Type III foci relative to the negative control.
<u>Results</u>	The test substance did not induce a statistically significant level of morphological transformation in BALB/3T3 cells.
Remarks	Relative to solvent control cell survival was 32%, 88% and 102% at 30, 20, and 10 ug/ml respectively. One spontaneous Type II but no Type III foci were observed in the solvent control. One Type II focus was observed at 30 ug/ml and one Type III focus was observed at 20 and 10 ug/ml each. The transformation frequencies were not statistically increased compared to the acetone control. The positive control induced 6 Type II and 14 Type III foci. Based on these results the negative and positive controls fulfilled the requirements for the determination of a valid test.
<u>Conclusions</u>	The test substance did not induce a statistically significant level of morphological transformation in BALB/3T3 cells.
<u>Data Quality</u>	Reliable without restriction (Klimisch Code)
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 8/7/00 (RTA-064)

Robust Summary 5-GenTox-11

<u>Test Substance</u>	
CAS #	CAS# 84605-29-8
Chemical Name	Phosphorodithioic acid, mixed O,O-bis (1,3-dimethylbutyl and iso-Pr) esters, zinc salts
Remarks	Test material purity not provided.
<u>Method</u>	
Method/Guideline followed	OECD Guideline 474
Test Type	Mammalian Erythrocyte Micronucleus Test
GLP (Y/N)	Y
Year (Study Performed)	1996
Species	Mouse
Strain	Swiss Albino Crl:CD-1 (ICR)BR
Route of administration	Intraperitoneal
Duration of test	Single dose followed by 72-hour evaluation period.
Doses/concentration levels	0, 7.13, 14.3 and 28.5 mg/kg
Dose volume	10 ml/kg
Sex	Males and females
Frequency of treatment	Once
Control and treatment groups	Peanut oil vehicle control: 15/sex; cyclophosphamide positive control: 60 mg/kg, 5/sex; 7.13 and 14.3 mg/kg: 15/sex; 28.3 mg/kg: 20/sex
Statistical methods	Animal to animal variability in spontaneous frequency of micronucleated polychromatic erythrocytes was evaluated in vehicle controls. Statistically significant differences were evaluated in the frequency of micronucleated polychromatic erythrocytes between treated groups and vehicle controls. NCE/PCE (normochromatic erythrocytes/polychromatic erythrocytes) ratios in treated and control groups were compared. Tests included Cochran-Armitage test for trend, a one-way analysis of variance and Dunnett's procedure.
Dose rangefinding study	Three rangefinding studies was conducted at the following dose levels: Study I: 554, 1802, 3049, 4296 and 5544 mg/kg Study II: 37.5, 62.5, 125 and 250 mg/kg Study III: 25, 12.5, 6.25, 3.13 and 1.0 mg/kg/day Mortality and physical observations were evaluated.
Remarks field for test conditions	All animals observed frequently for physiological or behavioral abnormalities on the day of dosing and periodically thereafter. Body weights taken on first day of the study prior to treatment. Five/sex from each treatment group and vehicle control group were sacrificed for bone marrow sampling 24, 48 and 72 hours post treatment. Positive controls sampled at 24 hours only. NCE/PCE ratio and %PCE of total erythrocytes were calculated by counting a total of ≥ 1000 erythrocytes/animal. A total of 1000 PCE /animal were evaluated for the presence of micronuclei. (Guideline calls for 2000/animal to be evaluated. This difference from the current guideline was not considered sufficient to effect the reliability of the study.)

<u>Results</u>	
Remarks	<p>During the first two dose rangefinding studies significant mortality was observed at all dose levels. In the third rangefinding study 1 high dose male died. Hypoactivity was observed in some animals at all dose levels greater than 1 mg/kg. Based on these data dose levels of 0, 7.13, 14.3 and 28.5 mg/kg were selected for the study.</p> <p>During the main study hypoactivity was observed in all dose groups during the first two hours post dosing. At 72 hours post dosing one male in the 7.13 mg/kg dose group was found dead.</p> <p>No statistically significant increases in micronucleated polychromatic erythrocytes over the levels observed in the vehicle controls were observed in either sex or at any harvest time point. All values for individual animals were within the expected range of micronucleated-PCE/1000 PCE expected for control animals. The variability in response observed in the treated animals was similar to that observed in the vehicle control. The positive control exhibited a statistically significant increase in micronuclei as expected. The test article did not induce a statistically significant change in the PCE:NCE ratio.</p>
<u>Conclusions</u>	Under the conditions of this study the test material did not induce micronuclei in bone marrow erythrocytes of mice.
<u>Data Quality</u>	Reliable without restriction (Klimisch Code)
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 7/17/00 (RTA-057)

Robust Summary 5-GenTox-12

<u>Test Substance</u>	
CAS #	CAS# 84605-29-8
Chemical Name	Phosphorodithioic acid, mixed O,O-bis (1,3-dimethylbutyl and iso-Pr) esters, zinc salts
Remarks	Test material purity not provided.
<u>Method</u>	
Method/Guideline followed	OECD Guideline 471
Test Type	Bacterial Reverse Mutation Assay
GLP (Y/N)	Y
Year (Study Performed)	1996
Test System	<i>Salmonella typhimurium</i> and <i>Escherichia Coli</i>
Strains Tested	<i>Salmonella typhimurium</i> tester strains TA98, TA100, TA1535, TA1537; <i>Escherichia Coli</i> tester strain WP2uvrA
Exposure Method	Plate incorporation
Test Substance Doses/concentration levels	Initial assay: <i>Salmonella</i> + (S9):25, 50, 100, 250, 1,000, and 5,000 ug/plate <i>Salmonella</i> - (S9): 10, 25, 50, 120, 600 and 3,000 ug/plate WP2uvrA + (S9): 100, 250, 500, 1,000, 5,000, and 10,000 ug/plate WP2uvrA - (S9): 25, 50, 100, 250, 1,250 and 6,500 ug/plate Confirmatory assay: <i>Salmonella</i> + (S9):100, 250, 500, 1,000, 2,500, and 5,000 ug/plate <i>Salmonella</i> - (S9): 50, 100, 250, 500, 1,000 and 3,000 ug/plate WP2uvrA + (S9): 100, 250, 500, 1,000, 5,000, and 10,000 ug/plate WP2uvrA - (S9): 100, 250, 500, 1,000, 2,000, and 6,500 ug/plate
Metabolic Activation	With and without (500 ul of 10% S9 fraction mix of livers of Aroclor 1254 pretreated Sprague Dawley rats)
Vehicle	Ethanol
Tester strain, activation status, Positive Controls and concentration level	TA98 +S9 benzo(a)pyrene 2.5 ug/plate TA98 -S9 2-nitroflourene 1.0 ug/plate TA100 +S9 2-aminoanthracene 2.5 ug/plate TA100 -S9 sodium azide 2.0 ug/plate TA1535 +S9 2-aminoanthracene 2.5 ug/plate TA1535 -S9 sodium azide 2.0 ug/plate TA1537 +S9 2-aminoanthracene 2.5 ug/plate TA1537 -S9 ICR-191 2.0 ug/plate WP2uvrA +S9 2-aminoanthracene 25.0 ug/plate WP2uvrA -S9 4-nitroquinoline-N-oxide 1.0 ug/plate
Vehicle Control	Ethanol
Statistical Analysis	Mean revertant colony count and standard deviation were determined for each dose point.
Dose Rangefinding Study	Conducted using tester strains TA100 and WP2uvrA and ten doses of test material ranging from 10.0 to 10,000 ug/plate, one plate/dose with (10% S9 homogenate/ml of S9 mix) and without metabolic activation. Cytotoxicity was evaluated.
S9 Optimization Study	Conducted using tester strains TA98 and TA100, and a cytotoxic dose level of test article (333 ug/plate) and four concentrations of S9 mix

	(5, 10, 20 and 80% S9 homogenate/ml of S9 mix). Cytotoxicity was evaluated.
Remarks field for test conditions	In the main study there were two treatment sets for each tester strain, with (+S9) and without (-S9) metabolic activation. Each of the tester strains was dosed with six concentrations of test substance, vehicle controls, and a positive control. Three plates/dose group/strain/treatment set were evaluated. The results of the initial assay were confirmed in a second independent experiment. 50 ul of test material, positive control or vehicle control were added to each plate along with 100 ul of tester strain, S9 mix (if needed) and 2.0 (with S9) or 2.5 ml (without S9) of top agar. This was overlaid onto the surface of 25 ml minimal bottom agar in a petri dish. Plates were incubated for 48 hours at 37°C. Plates that were not evaluated immediately were held at 5°C until evaluated. The condition of the bacterial background lawn was evaluated for cytotoxicity and test article precipitate.
<u>Results</u>	The test substance was not genotoxic in this assay with or without metabolic activation.
Remarks	<p>In the dose rangefinding study cytotoxicity was observed with tester strain TA100 at 667 ug/plate and above with metabolic activation and at 100 ug/plate and above without metabolic activation. With tester strain WP2uvrA cytotoxicity was observed at 10,000 ug/plate with activation and at 667 ug/plate and above without activation. Test article precipitate was observed on plates at 6670 ug/plate and above with tester strains TA100 and WP2uvrA without activation. With TA100 with metabolic activation precipitate was observed on plates at 1000 ug/plate and higher. With WP2uvrA with metabolic activation precipitate was observed at 3330 ug/plate and above. Based on these results dose levels outlined above (page 1, Test Substance Doses, Initial Assay) were selected.</p> <p>The S9 optimization study was performed using TA98 and TA100 with the highest non-cytotoxic dose of test article, (10,000 ug/plate) and four concentrations of S9 mix (5, 10, 20 and 80% S9 homogenate/ml of S9 mix). In the absence of any effect a 10% S9 mix was used in the mutagenicity study.</p> <p>In the initial and confirmatory mutagenicity assay all data were acceptable and no positive increases in the number of revertants/plate were observed with any of the tester strains with or without metabolic activation. Based on the results of the initial study the dilution factor between doses was reduced for the confirmatory study. The doses outlined above (page 1, Test Substance Doses, Confirmatory Assay) were utilized.</p> <p>Cytotoxicity was observed at ≥ 500 ug/plate with the <i>Salmonella</i> and WP2uvrA tester strains with and without activation. Test material participate was observed on plates at $\geq 3,000$ ug/plate.</p> <p>The positive control for each respective test strain exhibited at least a</p>

	3-fold increase (with or without S9) over the mean value of the vehicle control for a given strain, confirming the expected positive control response.
<u>Conclusions</u>	Under the conditions of this study, the test material was not mutagenic.
<u>Data Quality</u>	Reliable without restriction (Klimisch Code)
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 7/14/00 (RTA-052)

Robust Summary 5-GenTox-13

<u>Test Substance</u>	
CAS #	4259-15-8
Chemical Name	2-ethyl hexyl derivative
Remarks	Test material purity not provided.
<u>Method</u>	
Method/Guideline followed	Schechtman, L., Kouri, R., Progress in Genetic Toxicology, Elsevier/North-Holland Biomedical Press, New York, pp. 307-316 (1977).
Test Type	Morphological transformation of BALB/3T3 mouse embryo cells in the presence and absence of metabolic activation
GLP (Y/N)	Y
Year (Study Performed)	1982
Test System	BALB/3T3 mouse embryo cells
Exposure Method	Dilution
Test Substance Doses/concentration levels	Without metabolic activation: 8, 15, 30 ug/ml With metabolic activation Study A: 4, 5, 6 ug/ml With metabolic activation Study B: 6, 7, 8 ug/ml
Metabolic Activation	Yes (Aroclor-1254 treated rat liver homogenate). Each batch of +S9 was characterized by its ability to metabolize 2-aminoanthracene and benzo(a)pyrene to forms mutagenic to <i>s. typhimurium</i> .
Vehicle	The test material was solubilized in acetone and diluted to the appropriate concentration in complete Eagle's minimal essential medium supplemented with 10% fetal bovine serum, 1% L-glutamine and 2% penicillin-streptomycin. Solvent control plates were treated with acetone at the same concentration needed to expose the target cells to the highest dose of test article in complete medium.
Positive Control concentration level	Benzo(a)pyrene: 12.5 ug/ml used with metabolic activation. N-methyl-N'-nitro-N-nitrosoquandine: 0.5 ug/ml used without metabolic activation.
Statistical Analysis	The transforming potential of each treatment condition was compared to that of the solvent control using the Poisson distribution.
Test Substance Solubility	Test substance was solubilized in acetone.
Dose rangefinding study	Yes
Remarks field for test conditions	<p>Exponentially growing 3T3 clone A31-1 cells were seeded for an evaluation of cytotoxicity at 250 cells/culture in triplicate/condition, and at 1×10^4 cells/plate in 12-15 replicates for the determination of phenotypic transformation. Plates were incubated at 36° in a humidified atmosphere of 5% CO₂ in air for 20-24 hours. For activation assays cells were treated in suspension to a reaction mixture of NADP, NADH, NADPH, S-9 and test or control material prior to seeding. Cells were exposed to three concentrations of test article as well as to solvent and positive controls for 24 hours. Cells were then washed and the treated media replaced with untreated growth medium. After 7-10 days incubation, the concurrent cytotoxicity plates were fixed with methanol, stained with 10% Giemsa, and scored for colony formation. After 4-6 weeks incubation with twice weekly medium changes, the transformation plates were fixed with methanol, stained with 10% Giemsa, and scored for morphologically transformed Type II and Type III foci.</p> <p>The cytotoxic effects of each treatment condition were expressed relative to the</p>

	<p>solvent treated control (relative cloning efficiency). The transformation frequency for each treatment group was expressed as the number of transformed foci/surviving cell. For a valid test the cloning efficiency of the solvent control must be greater than or equal to 25%. The relative survival of cells exposed to the test article must fall within the range of 30-60% for one dose level and 60-90% for another dose level. The number of Type III foci in the negative control must not exceed one-focus/total replicate plates. The positive control must induce a significant ($p \leq 0.05$) number of Type III foci relative to the negative control.</p>
<u>Results</u>	<p>3T3 cell transforming activity was not observed under the conditions of this study in the absence of metabolic activation. 3T3 cell transforming activity was observed under the conditions of this study in the presence of metabolic activation.</p>
Remarks	<p>In the absence of metabolic activation (-S9), relative to solvent control, cell survival was 49%, 66% and 95% at 30, 15 and 8.0 ug/ml respectively. No Type II or Type III transformed foci were observed at any dose level tested. One spontaneous Type III focus was observed in the solvent control. The positive control induced 6 Type II and 17 Type III foci. Based on these results the negative and positive controls fulfilled the requirements for the determination of a valid test.</p> <p>In the presence of metabolic activation (+S9), relative to solvent control, cell survival was 65%, 79% and 95% at 6, 5 and 4 ug/ml respectively (Study A) and 0%, 0% and 55% at 8, 7, and 6 ug/ml (Study B). A repeat assay was performed in an attempt to obtain higher levels of toxicity. Type II and Type III foci were observed in the treated cultures as follows:</p> <p>Initial Assay (+S9) 4 ug/ml: 1 Type II; 3 Type III 5 ug/ml: 5 Type II; 4 Type III 6 ug/ml: 2 Type II; 1 Type III</p> <p>Repeat Assay (+S9) 6 ug/ml: 0 Type II; 2 Type III 7 ug/ml: toxic 8 ug/ml: toxic</p> <p>The transformation frequency of the 5 ug/ml dose group was statistically significantly greater than the solvent control.</p> <p>No spontaneous Type III foci were observed in the solvent control in the initial or repeat assay. The positive control induced 9 Type II and 6 Type III foci in the initial assay and 6 Type II and 7 Type III foci in the repeat assay. Based on these results the negative and positive controls fulfilled the requirements for the determination of a valid test.</p>

<u>Conclusions</u>	<p>3T3 cell transforming activity was not observed under the conditions of this study in the absence of metabolic activation.</p> <p>3T3 cell transforming activity was observed under the conditions of this study in the presence of metabolic activation.</p>
<u>Data Quality</u>	Reliable without restriction (Klimisch Code)
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 4/24/01 (RTA-)

Robust Summary 5-GenTox-14

<u>Test Substance</u>	
CAS #	84605-29-8
Chemical Name	Phosphorodithioic acid, mixed O,O-bis (1,3-dimethylbutyl and iso-Pr) esters, zinc salts
Remarks	Test material purity not provided.
<u>Method</u>	
Method/Guideline followed	Schechtman, L., Kouri, R., Progress in Genetic Toxicology, Elsevier/North-Holland Biomedical Press, New York, pp. 307-316 (1977).
Test Type	Morphological transformation of BALB/3T3 mouse embryo cells in the presence and absence of metabolic activation
GLP (Y/N)	Y
Year (Study Performed)	1982
Test System	BALB/3T3 mouse embryo cells
Exposure Method	Dilution
Test Substance Doses/concentration levels	Without metabolic activation: 16, 24 and 32 ug/ml With metabolic activation: 14, 16, 18 and 20 ug/ml
Metabolic Activation	Yes (Aroclor-1254 treated rat liver homogenate). Each batch of +S9 was characterized by its ability to metabolize 2-aminoanthracene and benzo(a)pyrene to forms mutagenic to <i>s. typhimurium</i> .
Vehicle	The test material was solubilized in acetone and diluted to the appropriate concentration in complete Eagle's minimal essential medium supplemented with 10% fetal bovine serum, 1% L-glutamine and 2% penicillin-streptomycin. Solvent control plates were treated with acetone at the same concentration needed to expose the target cells to the highest dose of test article in complete medium.
Positive Control concentration level	Benzo(a)pyrene: 12.5 ug/ml used with metabolic activation. N-methyl-N'-nitro-N-nitrosoquianidine: 0.5 ug/ml used without metabolic activation.
Statistical Analysis	The transforming potential of each treatment condition was compared to that of the solvent control using the Poisson distribution.
Test Substance Solubility	Test substance was solubilized in acetone.
Dose rangefinding study	Yes
Remarks field for test conditions	Exponentially growing 3T3 clone A31-1 cells were seeded for an evaluation of cytotoxicity at 250 cells/culture in triplicate/condition, and at 1×10^4 cells/plate in 12-15 replicates for the determination of phenotypic transformation. Plates were incubated at 36° in a humidified atmosphere of 5% CO ₂ in air for 20-24 hours. For activation assays cells were treated in suspension to a reaction mixture of NADP, NADH, NADPH, S-9 and test or control material prior to seeding. Cells were exposed to three concentrations of test article as well as to solvent and positive controls for 24 hours. Cells were then washed and the treated media replaced with untreated growth medium. After 7-10 days incubation, the concurrent cytotoxicity plates were fixed with methanol, stained with 10% Giemsa, and scored for colony formation. After 4-6 weeks incubation with twice weekly medium changes, the transformation plates were fixed with methanol, stained with 10% Giemsa, and scored for morphologically transformed Type II and Type III foci.

	<p>The cytotoxic effects of each treatment condition were expressed relative to the solvent treated control (relative cloning efficiency). The transformation frequency for each treatment group was expressed as the number of transformed foci/surviving cell. For a valid test the cloning efficiency of the solvent control must be greater than or equal to 25%. The relative survival of cells exposed to the test article must fall within the range of 30-60% for one dose level and 60-90% for another dose level. The number of Type III foci in the negative control must not exceed one-focus/total replicate plates. The positive control must induce a significant ($p \leq 0.05$) number of Type III foci relative to the negative control.</p>
<u>Results</u>	<p>3T3 cell transforming activity was not observed under the conditions of this study in the absence of metabolic activation. 3T3 cell transforming activity was observed under the conditions of this study in the presence of metabolic activation.</p>
Remarks	<p>In the absence of metabolic activation (-S9), relative to solvent control, cell survival was 15%, 46% and 95% at 32, 24 and 16 ug/ml respectively. One spontaneous Type III focus was observed in the solvent control. The positive control induced 20 Type II and 21 Type III foci. Based on these results the negative and positive controls fulfilled the requirements for the determination of a valid test. Type II and Type III foci were observed in the treated cultures as follows:</p> <p>Dose Level (-S9) 16 ug/ml: 0 Type II; 3 Type III 24 ug/ml: 0 Type II; 2 Type III 32 ug/ml: 0 Type II; 1 Type III</p> <p>The transformation frequencies of the test material treated groups were not statistically increased when compared to that of the solvent control.</p> <p>In the presence of metabolic activation (+S9), relative to solvent control, cell survival was 13%, 19%, 47% and 95% at 20, 18, 16 and 14 ug/ml respectively. Two Type II and one Type III spontaneous foci were observed in the solvent control. The positive control induced 5 Type II and 14 Type III foci. Based on these results the negative and positive controls fulfilled the requirements for the determination of a valid test. Type II and Type III foci were observed in the treated cultures as follows:</p> <p>Dose Level (+S9) 14 ug/ml: 2 Type II; 1 Type III 16 ug/ml: 0 Type II; 4 Type III 18 ug/ml: 3 Type II; 6 Type III 20 ug/ml: 2 Type II; 5 Type III</p> <p>The transformation frequencies of the test material treated groups at 18 and 20 ug/ml were statistically increased when compared to that of the solvent control.</p>

<u>Conclusions</u>	<p>3T3 cell transforming activity was not observed under the conditions of this study in the absence of metabolic activation.</p> <p>3T3 cell transforming activity was observed under the conditions of this study in the presence of metabolic activation.</p>
<u>Data Quality</u>	Reliable without restriction (Klimisch Code)
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 4/25/01 (RTA-)

Robust Summary 5-GenTox-15

<u>Test Substance</u>	
CAS #	113706-15-3
Chemical Name	Phosphorodithioic acid, secondary butyl and isooctyl) mixed esters, Zn Salts
Remarks	Test material purity not provided.
<u>Method</u>	
Method/Guideline followed	Schechtman, L., Kouri, R., Progress in Genetic Toxicology, Elsevier/North-Holland Biomedical Press, New York, pp. 307-316 (1977).
Test Type	Morphological transformation of BALB/3T3 mouse embryo cells in the absence of metabolic activation
GLP (Y/N)	Y
Year (Study Performed)	1982
Test System	BALB/3T3 mouse embryo cells
Exposure Method	Dilution
Test Substance Doses/concentration levels	18, 27, 36 ug/ml
Metabolic Activation	No
Vehicle	The test material was solubilized in acetone and diluted to the appropriate concentration in complete Eagle's minimal essential medium supplemented with 10% fetal bovine serum, 1% L-glutamine and 2% penicillin-streptomycin. Solvent control plates were treated with acetone at the same concentration needed to expose the target cells to the highest dose of test article in complete medium.
Positive Control concentration level	N-methyl-N'-nitro-N-nitrosoguanidine: 0.5 ug/ml
Statistical Analysis	The transforming potential of each treatment condition was compared to that of the solvent control using the Poisson distribution.
Test Substance Solubility	Test substance was solubilized in acetone.
Dose rangefinding study	Yes
Remarks field for test conditions	<p>Exponentially growing 3T3 clone A31-1 cells were seeded for an evaluation of cytotoxicity at 250 cells/culture in triplicate/condition, and at 1×10^4 cells/plate in 12-15 replicates for the determination of phenotypic transformation. Plates were incubated at 36° in a humidified atmosphere of 5% CO₂ in air for 20-24 hours. Cells were exposed to three concentrations of test article as well as to solvent and positive controls for 24 hours. Cells were then washed and the treated media replaced with untreated growth medium. After 7-10 days incubation, the concurrent cytotoxicity plates were fixed with methanol, stained with 10% Giemsa, and scored for colony formation. After 4-6 weeks incubation with twice weekly medium changes, the transformation plates were fixed with methanol, stained with 10% Giemsa, and scored for morphologically transformed Type II and Type III foci.</p> <p>The cytotoxic effects of each treatment condition were expressed relative to the solvent treated control (relative cloning efficiency). The transformation frequency for each treatment group was expressed as the number of transformed foci/surviving cell. For a valid test the cloning efficiency of the solvent control must be greater than or equal to 25%. The relative survival of cells exposed to the test article must fall within the range of 30-60% for one dose level and 60-90% for another dose level. The number of Type III foci in the negative control</p>

	must not exceed one-focus/total replicate plates. The positive control must induce a significant ($p \leq 0.05$) number of Type III foci relative to the negative control.
<u>Results</u>	3T3 cell transforming activity was observed under the conditions of this study in the absence of metabolic activation at extremely toxic doses. The Study Director concluded that the test material should be considered suspect for transforming activity in this assay.
<u>Remarks</u>	<p>In the absence of metabolic activation (-S9), relative to solvent control, cell survival was 5%, 49% and 89% at 36, 27 and 18 ug/ml, respectively. No spontaneous foci were observed in the solvent control. The positive control induced 16 Type II and 27 Type III foci. Based on these results the negative and positive controls fulfilled the requirements for the determination of a valid test.</p> <p>Type II and Type III foci were observed in the test material treated cultures as follows:</p> <p>Dose Level (-S9)</p> <p>18 ug/ml: 0 Type II; 1 Type III</p> <p>27 ug/ml: 1 Type II; 2 Type III</p> <p>36 ug/ml: 1 Type II; 3 Type III</p> <p>The transformation frequency of the 36 ug/ml dose group was statistically significantly greater than that of the solvent control.</p>
<u>Conclusions</u>	3T3 cell transforming activity was observed under the conditions of this study in the absence of metabolic activation at extremely toxic doses. The Study Director concluded that the test material should be considered suspect for transforming activity in this assay.
<u>Data Quality</u>	Reliable without restriction (Klimisch Code)
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 4/25/01 (RTA-)

Robust Summary 5-GenTox-16

<u>Test Substance</u>	
CAS #	68457-79-4
Chemical Name	Phosphorodithioic acid, mixed O,O-bis(iso-Bu and pentyl) esters, zinc salts
Remarks	Test material purity not provided.
<u>Method</u>	
Method/Guideline followed	Schechtman, L., Kouri, R., Progress in Genetic Toxicology, Elsevier/North-Holland Biomedical Press, New York, pp. 307-316 (1977).
Test Type	Morphological transformation of BALB/3T3 mouse embryo cells in the absence of metabolic activation
GLP (Y/N)	Y
Year (Study Performed)	1982
Test System	BALB/3T3 mouse embryo cells
Exposure Method	Dilution
Test Substance Doses/concentration levels	30, 45, 60 ug/ml
Metabolic Activation	No
Vehicle	The test material was solubilized in acetone and diluted to the appropriate concentration in complete Eagle's minimal essential medium supplemented with 10% fetal bovine serum, 1% L-glutamine and 2% penicillin-streptomycin. Solvent control plates were treated with acetone at the same concentration needed to expose the target cells to the highest dose of test article in complete medium.
Positive Control concentration level	N-methyl-N'-nitro-N-nitrosoguanidine: 0.5 ug/ml
Statistical Analysis	The transforming potential of each treatment condition was compared to that of the solvent control using the Poisson distribution.
Test Substance Solubility	Test substance was solubilized in acetone.
Dose rangefinding study	Yes
Remarks field for test conditions	<p>Exponentially growing 3T3 clone A31-1 cells were seeded for an evaluation of cytotoxicity at 250 cells/culture in triplicate/condition, and at 1×10^4 cells/plate in 12-15 replicates for the determination of phenotypic transformation. Plates were incubated at 36° in a humidified atmosphere of 5% CO₂ in air for 20-24 hours. Cells were exposed to three concentrations of test article as well as to solvent and positive controls for 24 hours. Cells were then washed and the treated media replaced with untreated growth medium. After 7-10 days incubation, the concurrent cytotoxicity plates were fixed with methanol, stained with 10% Giemsa, and scored for colony formation. After 4-6 weeks incubation with twice weekly medium changes, the transformation plates were fixed with methanol, stained with 10% Giemsa, and scored for morphologically transformed Type II and Type III foci.</p> <p>The cytotoxic effects of each treatment condition were expressed relative to the solvent treated control (relative cloning efficiency). The transformation frequency for each treatment group was expressed as the number of transformed foci/surviving cell. For a valid test the cloning efficiency of the solvent control must be greater than or equal to 25%. The relative survival of cells exposed to the test article must fall within the range of 30-60% for one dose level and 60-90% for another dose level. The number of Type III foci in the negative control</p>

	must not exceed one-focus/total replicate plates. The positive control must induce a significant ($p \leq 0.05$) number of Type III foci relative to the negative control.
<u>Results</u>	Substantial, though not statistically significant, 3T3 cell transforming activity was observed, under the conditions of this study, in the absence of metabolic activation at moderate to non-toxic dose levels. The Study Director concluded that this test material should be considered suspect of transformation activity in this assay.
<u>Remarks</u>	<p>In the absence of metabolic activation (-S9), relative to solvent control, cell survival was 1%, 31% and 67% at 60, 45 and 30 ug/ml respectively. One spontaneous Type III focus was observed in the solvent control. The positive control induced 21 Type II and 26 Type III foci. Based on these results the negative and positive controls fulfilled the requirements for the determination of a valid test.</p> <p>Type II and Type III foci were observed in the test material treated cultures as follows: Dose Level (-S9) 30 ug/ml: 1 Type II; 0 Type III 45 ug/ml: 1 Type II; 4 Type III 60 ug/ml: 1 Type II; 0 Type III</p>
<u>Conclusions</u>	Substantial, though not statistically significant, 3T3 cell transforming activity was observed, under the conditions of this study, in the absence of metabolic activation at moderate to non-toxic dose levels. The Study Director concluded that this test material should be considered suspect of transformation activity in this assay.
<u>Data Quality</u>	Reliable without restriction (Klimisch Code)
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 4/25/01 (RTA-)

Robust Summary 5-GenTox-17

<u>Test Substance</u>	
CAS #	4259-15-8
Chemical Name	2-ethyl hexyl derivative
Remarks	Test material purity not provided.
<u>Method</u>	
Method/Guideline followed	Consistent With OECD Guideline 476
Test Type	Mouse Lymphoma Mutagenicity Screen
GLP (Y/N)	Y
Year (Study Performed)	1983
Test System	L5178Y mouse lymphoma cells Clone 3.7.2C
Culture Preparation and Maintenance	L5178Y cells, which were actively growing in culture, were cleansed. Three ml of THMG (Thymidine, Hypoxanthine, Methotrexate, Glycine) were added to 100 ml cell suspension containing 0.1×10^6 cells/ml. Culture was gassed with 5% CO ₂ in air and incubated at 37°C at 125 rpm for 24 hours. After 24 hours the THMG was removed and the cells were rinsed in 20 ml of Fisher's Media with 0.1% Pluronic with 10% heat inactivated serum (F10P) and reinstated in culture at 3×10^4 cells/ml in 100 ml of F10P plus 1 ml of THG stock solution. Cell population density of the prepared cultures was determined by adding a 1 ml sample of cells to 9 ml of 0.1% trypsin, incubating at 37°C for 10 minutes, and making three counts/sample with an electronic cell counter. A cell suspension containing 1×10^6 cells/ml was then prepared and 6 ml aliquots were dispensed to polypropylene centrifuge tubes.
Exposure Method	Dilution
Test Substance Doses/concentration levels	Without metabolic activation: 0.0089, 0.0067, 0.005, 0.0038, 0.0028, 0.0021, 0.0016, 0.0012, 0.00089, 0.00067 ul/ml. With metabolic activation: Assay A: 0.021, 0.016, 0.012, 0.0089, 0.0067, 0.005, 0.0038, 0.0028, 0.0021, or 0.0016 ul/ml. With metabolic activation: Assay B: 0.022, 0.021, 0.020, 0.019, 0.018, or 0.017 ul/ml. With metabolic activation: Assay C: 0.021, 0.018, or 0.017 ul/ml. With metabolic activation: Assay D: 0.021, 0.016, 0.012, 0.0089, 0.0067, 0.005, 0.0038, 0.0028, 0.0021, or 0.0016 ul/ml.
Metabolic Activation	Aroclor 1242/1254 induced rat liver
Vehicle	Acetone
Positive Control concentration levels by activation status	With activation: 7,12-dimethylbenzanthracene (DMBA) 7.5 and 5 ug/mL Without activation: ethylmethanesulfonate (EMS) 1.0 and 0.5 ul/mL
Statistical Analysis	Means and standard deviations were determined. Plates were scored for total number of colonies/plate. Three counts/plate were made using an automatic colony counter. The median count was recorded. Plates were counted by hand if necessary. Mutation frequency was determined by dividing the average number of colonies in the treated plates by the average number of colonies ($\times 10^4$) in the corresponding vehicle control plates and multiplying by two. By comparing the mutation frequency of the treated plates to that of the control plates, the presence of a significant level of mutagenic activity can be detected.
Test Substance Solubility	Test substance solubility in the vehicle was determined during a solubility test that compared the solubility of the test material in acetone, DMSO, ethanol and

	water. Acetone was selected as the appropriate vehicle.
Toxicity Determination	<p>A preliminary toxicity test with and without S-9 activation was conducted. Cultures were initiated by seeding one tube/dose and two/solvent control with 6 ml of cell suspension from a common pool containing 1×10^6 cells/ml. The test material was solubilized and diluted for testing at 10, 5, 1, 0.5, 0.1, 0.05, 0.01, 0.005 and 0.001 ul/ml with S-9. In the absence of S-9, the test material was prepared at 100, 10, 5, 1, 0.5, 0.1, 0.05, 0.01, 0.005 and 0.001 ul/ml. The test material was added to each culture tube. Four ml of Fishers media or S-9 activation mixture were added to each tube. The tubes were gassed with 5% CO₂ in air and incubated at 37°C at 25 rpm for 24 hours. Cultures were prepared under amber light and kept in darkness during incubation. After 4 hours the test material was removed by centrifuging the cells at 1000 rpm for 10 minutes and decanting. The cells were washed twice in 10 ml F10P, resuspended in 20 ml of F10P, gassed with 5% CO₂ in air and replaced on the mixer.</p> <p>Test material toxicity was determined by comparing cell population growth at each dose level with that of the solvent controls. Cell population density was determined 24 and 48 hours after the initial exposure to the test material by removing 1 ml samples from each culture, making 1:10 dilutions in 0.1% trypsin, incubating at 37°C for 10 minutes and counting the samples with an electronic cell counter.</p>
Mutagenicity Assay (Remarks field for test conditions)	<p>This study was conducted prior to the development of OECD Test Guideline 476. This study deviates from this guideline in that colony sizing was not performed. This deviation from the guideline was not considered sufficient to invalidate this study.</p> <p>Based on the toxicity determination the test material was prepared so that the highest concentration was 100% toxic. The test material was solubilized and serial dilutions were carried out. Dose solutions were produced and cultures were treated in triplicate. The test material was added to the cultures along with S-9, as appropriate, in amounts at which the final solvent concentration was nontoxic to the cells. Final cell suspensions were 0.6×10^6 cells/ml. In the mutagenicity study there were two treatment sets for each concentration of test substance, with (+S9) and without (-S9) metabolic activation. DMBA (positive control) was tested with activation and EMS (positive control) was tested without activation.</p> <p>All tubes were gassed with 5% CO₂ in air and incubated at 37°C at 25 rpm for 4 hours. Cultures were prepared under amber light and kept in darkness during incubation. After 4 hours the test material was removed by centrifuging the cells at 1000 rpm for 10 minutes and decanting. The cells were washed twice in 10 ml F10P, resuspended in 20 ml of F10P, gassed with 5% CO₂ in air and replaced on the mixer at 37°C for two days. Cell population adjustments were made at 24 and 48 hours to yield a cell population of 0.3×10^6 cells/ml. The cells were then plated in a restrictive media containing 3 ug/ml trifluorothymidine (TFT) which allows TK⁻ cells to grow. Cells were also plated in a non-restrictive media that indicated cell viability.</p> <p>Plates were incubated at 37°C in a humidified 5% CO₂ atmosphere for 10-12 days. Following incubation all plates were scored for total number of colonies/plate. Three counts/plate were made on an automatic colony counter</p>

	<p>and the median count was recorded. The frequency of mutation by dose was determined by comparing the average number of colonies in the mutagenicity plates to the average number of colonies in the corresponding viability plates.</p> <p>For the study to be acceptable the following criteria must be met: mutation frequency of positive controls with or without activation should be twice that of the solvent control; the negative control spontaneous mutation frequency should be in the range of 0.2 to 1.0/10⁴ cells; negative control plating efficiency should be at or above 50%.</p> <p>The following criteria were used as guidelines in judging the significance of test material activity: Positive – if there is a positive dose response and one or more of the three highest dose levels exhibit a mutation frequency two-fold greater than background. Equivocal – no dose response but any one or more dose levels exhibit a 2x increase in mutation frequency over background. Negative - no dose response and none of the dose levels exhibit a 2x increase in mutation frequency over background.</p>
<u>Results</u>	The test substance was not mutagenic in this assay without metabolic activation and produced an equivocal response in the presence of metabolic activation.
Remarks	<p>Nonactivated cultures were cloned over a range of concentrations that produced 35 to 98% total growth. The S-9 cultures were cloned over a range of test article concentrations that produced 3 to 100% total growth.</p> <p>None of the nonactivated cultures exhibited mutant frequencies that were significantly greater than the mean mutant frequency of the solvent controls.</p> <p>The highest dose tested in the S-9 activated cultures exhibited a mutation frequency that was more than 2X the mean mutation frequency of the solvent controls. Based on this result three additional assays were conducted as follow-up studies with S-9 activation. In the first +S9 repeat assay total growth ranged from 3 to 71%. Some contamination was present and complete results were obtained from 11 of 18 cultures. These eleven cultures exhibited mutation frequencies that were significantly greater than the mutation frequency of the solvent control (2.2-9.4X solvent control). This assay was repeated due to the contamination and an erratic dose-response. In the second +S9 repeat assay total growth ranged from 3 to 44%. These cultures exhibited mutation frequencies that were significantly greater than the mutation frequency of the solvent control (3.1-11.3X solvent control). In the third +S9 repeat assay a second lot of test material was used. Total growth ranged from 27 to 96%. None of These cultures exhibited mutation frequencies that were significantly greater than the mutation frequency of the solvent control. However, a dose dependent increase in mutation frequency was noted (1.0-1.9X solvent control). Based on the results of this series of experiments it was concluded by the Study Director that the test material produced an equivocal response in the presences of metabolic activation.</p> <p>Positive and vehicle control group responses were appropriate and met the criteria outlined above.</p>

<u>Conclusions</u>	The test substance was not mutagenic in this assay without metabolic activation and produced an equivocal response in the presence of metabolic activation.
<u>Data Quality</u>	Reliable without restriction (Klimisch Code)
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 4/19/01 (RTA-0)

Robust Summary 5-GenTox-18

<u>Test Substance</u>	
CAS #	26566-95-0
Chemical Name	Phosphorodithioic acid, mixed 0-(2-ethylhexyl)- O-(2-methylpropyl) esters, zinc salts
Remarks	Test material purity not provided.
<u>Method</u>	
Method/Guideline followed	Consistent With OECD Guideline 476
Test Type	Mouse Lymphoma Mutagenicity Screen
GLP (Y/N)	Y
Year (Study Performed)	1983
Test System	L5178Y mouse lymphoma cells Clone 3.7.2C
Culture Preparation and Maintenance	L5178Y cells, which were actively growing in culture, were cleansed. Three ml of THMG (Thymidine, Hypoxanthine, Methotrexate, Glycine) were added to 100 ml cell suspension containing 0.1×10^6 cells/ml. Culture was gassed with 5% CO ₂ in air and incubated at 37°C at 125 rpm for 24 hours. After 24 hours the THMG was removed and the cells were rinsed in 20 ml of Fisher's Media with 0.1% Pluronic with 10% heat inactivated serum (F10P) and reinstated in culture at 3×10^4 cells/ml in 100 ml of F10P plus 1 ml of THG stock solution. Cell population density of the prepared cultures was determined by adding a 1 ml sample of cells to 9 ml of 0.1% trypsin, incubating at 37°C for 10 minutes, and making three counts/sample with an electronic cell counter. A cell suspension containing 1×10^6 cells/ml was then prepared and 6 ml aliquots were dispensed to polypropylene centrifuge tubes.
Exposure Method	Dilution
Test Substance Doses/concentration levels	Without metabolic activation: 0.0089, 0.0067, 0.005, 0.0038, 0.0028, 0.0021, 0.0016, 0.0012, 0.00089, 0.00067 ul/ml. With metabolic activation: 0.021, 0.016, 0.012, 0.0089, 0.0067, 0.005, 0.0038, 0.0028, 0.0021, or 0.0016 ul/ml.
Metabolic Activation	Aroclor 1242/1254 induced rat liver
Vehicle	Acetone
Positive Control concentration levels by activation status	With activation: 7,12-dimethylbenzanthracene (DMBA) 7.5 and 5 ug/mL Without activation: ethylmethanesulfonate (EMS) 1.0 and 0.5 ul/mL
Statistical Analysis	Means and standard deviations were determined. Plates were scored for total number of colonies/plate. Three counts/plate were made using an automatic colony counter. The median count was recorded. Plates were counted by hand if necessary. Mutation frequency was determined by dividing the average number of colonies in the treated plates by the average number of colonies ($\times 10^4$) in the corresponding vehicle control plates and multiplying by two. By comparing the mutation frequency of the treated plates to that of the control plates, the presence of a significant level of mutagenic activity can be detected.
Test Substance Solubility	Test substance solubility in the vehicle was determined during a solubility test that compared the solubility of the test material in acetone, DMSO, ethanol and water. Acetone was selected as the appropriate vehicle.

Toxicity Determination	<p>A preliminary toxicity test with and without S-9 activation was conducted. Cultures were initiated by seeding one tube/dose and two/solvent control with 6 ml of cell suspension from a common pool containing 1×10^6 cells/ml. The test material was solubilized and diluted for testing at 10, 5, 1, 0.5, 0.1, 0.05, 0.01, 0.005 and 0.001 ul/ml with S-9. In the absence of S-9, the test material was prepared at 100, 10, 5, 1, 0.5, 0.1, 0.05, 0.01, 0.005 and 0.001 ul/ml. The test material was added to each culture tube. Four ml of Fishers media or S-9 activation mixture were added to each tube. The tubes were gassed with 5% CO₂ in air and incubated at 37°C at 25 rpm for 24 hours. Cultures were prepared under amber light and kept in darkness during incubation. After 4 hours the test material was removed by centrifuging the cells at 1000 rpm for 10 minutes and decanting. The cells were washed twice in 10 ml F10P, resuspended in 20 ml of F10P, gassed with 5% CO₂ in air and replaced on the mixer.</p> <p>Test material toxicity was determined by comparing cell population growth at each dose level with that of the solvent controls. Cell population density was determined 24 and 48 hours after the initial exposure to the test material by removing 1 ml samples from each culture, making 1:10 dilutions in 0.1% trypsin, incubating at 37°C for 10 minutes and counting the samples with an electronic cell counter.</p>
Mutagenicity Assay (Remarks field for test conditions)	<p>This study was conducted prior to the development of OECD Test Guideline 476. This study deviates from this guideline in that colony sizing was not performed. This deviation from the guideline was not considered sufficient to invalidate this study.</p> <p>Based on the toxicity determination the test material was prepared so that the highest concentration was 100% toxic. The test material was solubilized and serial dilutions were carried out. Dose solutions were produced and cultures were treated in triplicate. The test material was added to the cultures along with S-9, as appropriate, in amounts at which the final solvent concentration was nontoxic to the cells. Final cell suspensions were 0.6×10^6 cells/ml. In the mutagenicity study there were two treatment sets for each concentration of test substance, with (+S9) and without (-S9) metabolic activation. DMBA (positive control) was tested with activation and EMS (positive control) was tested without activation.</p> <p>All tubes were gassed with 5% CO₂ in air and incubated at 37°C at 25 rpm for 4 hours. Cultures were prepared under amber light and kept in darkness during incubation. After 4 hours the test material was removed by centrifuging the cells at 1000 rpm for 10 minutes and decanting. The cells were washed twice in 10 ml F10P, resuspended in 20 ml of F10P, gassed with 5% CO₂ in air and replaced on the mixer at 37°C for two days. Cell population adjustments were made at 24 and 48 hours to yield a cell population of 0.3×10^6 cells/ml. The cells were then plated in a restrictive media containing 3 ug/ml trifluorothymidine (TFT) which allows TK⁻ cells to grow. Cells were also plated in a non-restrictive media that indicated cell viability.</p> <p>Plates were incubated at 37°C in a humidified 5% CO₂ atmosphere for 10-12 days. Following incubation all plates were scored for total number of colonies/plate. Three counts/plate were made on an automatic colony counter</p>

	<p>and the median count was recorded. The frequency of mutation by dose was determined by comparing the average number of colonies in the mutagenicity plates to the average number of colonies in the corresponding viability plates.</p> <p>For the study to be acceptable the following criteria must be met: mutation frequency of positive controls with or without activation should be twice that of the solvent control; the negative control spontaneous mutation frequency should be in the range of 0.2 to 1.0/10⁴ cells; negative control plating efficiency should be at or above 50%.</p> <p>The following criteria were used as guidelines in judging the significance of test material activity: Positive – if there is a positive dose response and one or more of the three highest dose levels exhibit a mutation frequency two-fold greater than background. Equivocal – no dose response but any one or more dose levels exhibit a 2x increase in mutation frequency over background. Negative - no dose response and none of the dose levels exhibit a 2x increase in mutation frequency over background.</p>
<u>Results</u>	The test substance was not mutagenic in this assay without metabolic activation and produced an equivocal response in the presence of metabolic activation.
Remarks	<p>Nonactivated cultures were cloned over a range of concentrations that produced 11 to 98% total growth. The S-9 cultures were cloned over a range of test article concentrations that produced 59 to 101% total growth.</p> <p>None of the nonactivated cultures exhibited mutant frequencies that were significantly greater than the mean mutant frequency of the solvent controls.</p> <p>One +S9 culture (0.021 ul/ml) that was cloned exhibited a mutation frequency that was 2X the mean mutant frequency of the solvent controls. The total growth of this culture was 59%. None of the remaining +S9 cultures that were cloned exhibited mutation frequencies that were significantly greater than the mean mutation frequency of the solvent controls. The total growth of these cultures ranged from 78 to 101%.</p> <p>Based on these results it was concluded by the Study Director that the test material produced an equivocal response in the presences of metabolic activation.</p> <p>Positive and vehicle control group responses were appropriate and met the criteria outlined above.</p>
<u>Conclusions</u>	The test substance was not mutagenic in this assay without metabolic activation and produced an equivocal response in the presence of metabolic activation.
<u>Data Quality</u>	Reliable without restriction (Klimisch Code)
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 4/20/01 (RTA-0)

Robust Summary 5-GenTox-19

<u>Test Substance</u>	
CAS #	113706-15-3
Chemical Name	Phosphorodithioic acid, secondary butyl and isooctyl) mixed esters, Zn Salts
Remarks	Test material purity not provided.
<u>Method</u>	
Method/Guideline followed	Consistent With OECD Guideline 476
Test Type	Mouse Lymphoma Mutagenicity Screen
GLP (Y/N)	Y
Year (Study Performed)	1983
Test System	L5178Y mouse lymphoma cells Clone 3.7.2C
Culture Preparation and Maintenance	L5178Y cells, which were actively growing in culture, were cleansed. Three ml of THMG (Thymidine, Hypoxanthine, Methotrexate, Glycine) were added to 100 ml cell suspension containing 0.1×10^6 cells/ml. Culture was gassed with 5% CO ₂ in air and incubated at 37°C at 125 rpm for 24 hours. After 24 hours the THMG was removed and the cells were rinsed in 20 ml of Fisher's Media with 0.1% Pluronic with 10% heat inactivated serum (F10P) and reinstated in culture at 3×10^4 cells/ml in 100 ml of F10P plus 1 ml of THG stock solution. Cell population density of the prepared cultures was determined by adding a 1 ml sample of cells to 9 ml of 0.1% trypsin, incubating at 37°C for 10 minutes, and making three counts/sample with an electronic cell counter. A cell suspension containing 1×10^6 cells/ml was then prepared and 6 ml aliquots were dispensed to polypropylene centrifuge tubes.
Exposure Method	Dilution
Test Substance Doses/concentration levels	Without metabolic activation: 0.024, 0.018, 0.013, 0.010, 0.0075, 0.0042, 0.0032, 0.0024 or 0.0018 ul/ml. With metabolic activation: 0.061, 0.049, 0.036, 0.023 or 0.01ul/ml.
Metabolic Activation	Aroclor 1242/1254 induced rat liver
Vehicle	Acetone
Positive Control concentration levels by activation status	With activation: 7,12-dimethylbenzanthracene (DMBA) 7.5 and 5 ug/mL Without activation: ethylmethanesulfonate (EMS) 1.0 and 0.5 ul/mL
Statistical Analysis	Means and standard deviations were determined. Plates were scored for total number of colonies/plate. Three counts/plate were made using an automatic colony counter. The median count was recorded. Plates were counted by hand if necessary. Mutation frequency was determined by dividing the average number of colonies in the treated plates by the average number of colonies ($\times 10^4$) in the corresponding vehicle control plates and multiplying by two. By comparing the mutation frequency of the treated plates to that of the control plates, the presence of a significant level of mutagenic activity can be detected.
Test Substance Solubility	Test substance solubility in the vehicle was determined during a solubility test that compared the solubility of the test material in acetone, DMSO, ethanol and water. Acetone was selected as the appropriate vehicle.

Toxicity Determination	<p>A preliminary toxicity test with and without S-9 activation was conducted. Cultures were initiated by seeding one tube/dose and two/solvent control with 6 ml of cell suspension from a common pool containing 1×10^6 cells/ml. The test material was solubilized and diluted for testing at 10, 5, 1, 0.5, 0.1, 0.05, 0.01, 0.005 and 0.001 ul/ml with S-9. In the absence of S-9, the test material was prepared at 100, 10, 1, 0.1, 0.01 and 0.001 ul/ml. The test material was added to each culture tube. Four ml of Fishers media or S-9 activation mixture were added to each tube. The tubes were gassed with 5% CO₂ in air and incubated at 37°C at 25 rpm for 24 hours. Cultures were prepared under amber light and kept in darkness during incubation. After 4 hours the test material was removed by centrifuging the cells at 1000 rpm for 10 minutes and decanting. The cells were washed twice in 10 ml F10P, resuspended in 20 ml of F10P, gassed with 5% CO₂ in air and replaced on the mixer.</p> <p>Test material toxicity was determined by comparing cell population growth at each dose level with that of the solvent controls. Cell population density was determined 24 and 48 hours after the initial exposure to the test material by removing 1 ml samples from each culture, making 1:10 dilutions in 0.1% trypsin, incubating at 37°C for 10 minutes and counting the samples with an electronic cell counter.</p>
Mutagenicity Assay (Remarks field for test conditions)	<p>This study was conducted prior to the development of OECD Test Guideline 476. This study deviates from this guideline in that colony sizing was not performed. This deviation from the guideline was not considered sufficient to invalidate this study.</p> <p>Based on the toxicity determination the test material was prepared so that the highest concentration was 100% toxic. The test material was solubilized and serial dilutions were carried out. Dose solutions were produced and cultures were treated in triplicate. The test material was added to the cultures along with S-9, as appropriate, in amounts at which the final solvent concentration was nontoxic to the cells. Final cell suspensions were 0.6×10^6 cells/ml. In the mutagenicity study there were two treatment sets for each concentration of test substance, with (+S9) and without (-S9) metabolic activation. DMBA (positive control) was tested with activation and EMS (positive control) was tested without activation.</p> <p>All tubes were gassed with 5% CO₂ in air and incubated at 37°C at 25 rpm for 4 hours. Cultures were prepared under amber light and kept in darkness during incubation. After 4 hours the test material was removed by centrifuging the cells at 1000 rpm for 10 minutes and decanting. The cells were washed twice in 10 ml F10P, resuspended in 20 ml of F10P, gassed with 5% CO₂ in air and replaced on the mixer at 37°C for two days. Cell population adjustments were made at 24 and 48 hours to yield a cell population of 0.3×10^6 cells/ml. The cells were then plated in a restrictive media containing 3 ug/ml trifluorothymidine (TFT) which allows TK⁻ cells to grow. Cells were also plated in a non-restrictive media that indicated cell viability.</p> <p>Plates were incubated at 37°C in a humidified 5% CO₂ atmosphere for 10-12 days. Following incubation all plates were scored for total number of colonies/plate. Three counts/plate were made on an automatic colony counter and the median count was recorded. The frequency of mutation by dose was determined by comparing the average number of colonies in the mutagenicity</p>

	<p>plates to the average number of colonies in the corresponding viability plates.</p> <p>For the study to be acceptable the following criteria must be met: mutation frequency of positive controls with or without activation should be twice that of the solvent control; the negative control spontaneous mutation frequency should be in the range of 0.2 to 1.0/10⁴ cells; negative control plating efficiency should be at or above 50%.</p> <p>The following criteria were used as guidelines in judging the significance of test material activity: Positive – if there is a positive dose response and one or more of the three highest dose levels exhibit a mutation frequency two-fold greater than background. Equivocal – no dose response but any one or more dose levels exhibit a 2x increase in mutation frequency over background. Negative - no dose response and none of the dose levels exhibit a 2x increase in mutation frequency over background.</p>
<u>Results</u>	The test substance was not mutagenic in this assay without metabolic activation and produced a positive response in the presence of metabolic activation.
Remarks	<p>Nonactivated cultures were cloned over a range of concentrations that produced 2 to 97% total growth. The S-9 cultures were cloned over a range of test article concentrations that produced 8 to 95% total growth.</p> <p>One of the nonactivated cultures (0.024 ul/ml) that was cloned exhibited a mutation frequency that was 2.8X the mean mutation frequency of the solvent controls. The total growth of this culture was 2%. None of the remaining cultures exhibited mutation frequencies that were significantly greater than the mean mutant frequency of the solvent controls. The total growth of these cultures ranged from 19 to 97%. The Study Director did not consider this single culture significant since mutation frequencies observed at such highly toxic levels may be due to epigenetic events.</p> <p>The +S9 cultures treated at 0.061, 0.049, 0.036, 0.023 ul/ml exhibited mutation frequencies that ranged from 13.2 to 2.0 times the mean mutant frequency of the solvent controls. The total growth of these cultures ranged from 8 to 84%. None of the remaining +S9 cultures that were cloned exhibited mutation frequencies that were significantly greater than the mean mutation frequency of the solvent controls. The total growth of these cultures ranged from 86 to 95%.</p> <p>Based on these results it was concluded by the Study Director that the test material produced a positive response in the presences of metabolic activation.</p> <p>Positive and vehicle control group responses were appropriate and met the criteria outlined above.</p>
<u>Conclusions</u>	The test substance was not mutagenic in this assay without metabolic activation and produced a positive response in the presence of metabolic activation.
<u>Data Quality</u>	Reliable without restriction (Klimisch Code)
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 4/23/01 (RTA-0)

Robust Summary 5-GenTox-20

<u>Test Substance</u>	
CAS #	68457-79-4
Chemical Name	Phosphorodithioic acid, mixed O,O-bis(iso-Bu and pentyl) esters, zinc salts
Remarks	Test material purity not provided.
<u>Method</u>	
Method/Guideline followed	Consistent With OECD Guideline 476
Test Type	Mouse Lymphoma Mutagenicity Screen
GLP (Y/N)	Y
Year (Study Performed)	1983
Test System	L5178Y mouse lymphoma cells Clone 3.7.2C
Culture Preparation and Maintenance	L5178Y cells, which were actively growing in culture, were cleansed. Three ml of THMG (Thymidine, Hypoxanthine, Methotrexate, Glycine) were added to 100 ml cell suspension containing 0.1×10^6 cells/ml. Culture was gassed with 5% CO ₂ in air and incubated at 37°C at 125 rpm for 24 hours. After 24 hours the THMG was removed and the cells were rinsed in 20 ml of Fisher's Media with 0.1% Pluronic with 10% heat inactivated serum (F10P) and reinstated in culture at 3×10^4 cells/ml in 100 ml of F10P plus 1 ml of THG stock solution. Cell population density of the prepared cultures was determined by adding a 1 ml sample of cells to 9 ml of 0.1% trypsin, incubating at 37°C for 10 minutes, and making three counts/sample with an electronic cell counter. A cell suspension containing 1×10^6 cells/ml was then prepared and 6 ml aliquots were dispensed to polypropylene centrifuge tubes.
Exposure Method	Dilution
Test Substance Doses/concentration levels	Without metabolic activation: 0.013, 0.010, 0.0075, 0.0056, 0.0042, 0.0032, 0.0024, 0.0018 or 0.0013 ul/ml. With metabolic activation: 0.024, 0.018, 0.013, 0.010, 0.0075, 0.0056, 0.0042, 0.0032, 0.0024 or 0.0018 ul/ml.
Metabolic Activation	Aroclor 1242/1254 induced rat liver
Vehicle	Acetone
Positive Control concentration levels by activation status	With activation: 7,12-dimethylbenzanthracene (DMBA) 7.5 and 5 ug/mL Without activation: ethylmethanesulfonate (EMS) 1.0 and 0.5 ul/mL
Statistical Analysis	Means and standard deviations were determined. Plates were scored for total number of colonies/plate. Three counts/plate were made using an automatic colony counter. The median count was recorded. Plates were counted by hand if necessary. Mutation frequency was determined by dividing the average number of colonies in the treated plates by the average number of colonies ($\times 10^4$) in the corresponding vehicle control plates and multiplying by two. By comparing the mutation frequency of the treated plates to that of the control plates, the presence of a significant level of mutagenic activity can be detected.
Test Substance Solubility	Test substance solubility in the vehicle was determined during a solubility test that compared the solubility of the test material in acetone, DMSO, ethanol and water. Acetone was selected as the appropriate vehicle.

Toxicity Determination	<p>A preliminary toxicity test with and without S-9 activation was conducted. Cultures were initiated by seeding one tube/dose and two/solvent control with 6 ml of cell suspension from a common pool containing 1×10^6 cells/ml. The test material was solubilized and diluted for testing at 10, 5, 1, 0.5, 0.1, 0.05, 0.01, 0.005 and 0.001 ul/ml with S-9. In the absence of S-9, the test material was prepared at 100, 10, 1, 0.1, 0.01 and 0.001 ul/ml. The test material was added to each culture tube. Four ml of Fishers media or S-9 activation mixture were added to each tube. The tubes were gassed with 5% CO₂ in air and incubated at 37°C at 25 rpm for 24 hours. Cultures were prepared under amber light and kept in darkness during incubation. After 4 hours the test material was removed by centrifuging the cells at 1000 rpm for 10 minutes and decanting. The cells were washed twice in 10 ml F10P, resuspended in 20 ml of F10P, gassed with 5% CO₂ in air and replaced on the mixer.</p> <p>Test material toxicity was determined by comparing cell population growth at each dose level with that of the solvent controls. Cell population density was determined 24 and 48 hours after the initial exposure to the test material by removing 1 ml samples from each culture, making 1:10 dilutions in 0.1% trypsin, incubating at 37°C for 10 minutes and counting the samples with an electronic cell counter.</p>
Mutagenicity Assay (Remarks field for test conditions)	<p>This study was conducted prior to the development of OECD Test Guideline 476. This study deviates from this guideline in that colony sizing was not performed. This deviation from the guideline was not considered sufficient to invalidate this study.</p> <p>Based on the toxicity determination the test material was prepared so that the highest concentration was 100% toxic. The test material was solubilized and serial dilutions were carried out. Dose solutions were produced and cultures were treated in triplicate. The test material was added to the cultures along with S-9, as appropriate, in amounts at which the final solvent concentration was nontoxic to the cells. Final cell suspensions were 0.6×10^6 cells/ml. In the mutagenicity study there were two treatment sets for each concentration of test substance, with (+S9) and without (-S9) metabolic activation. DMBA (positive control) was tested with activation and EMS (positive control) was tested without activation.</p> <p>All tubes were gassed with 5% CO₂ in air and incubated at 37°C at 25 rpm for 4 hours. Cultures were prepared under amber light and kept in darkness during incubation. After 4 hours the test material was removed by centrifuging the cells at 1000 rpm for 10 minutes and decanting. The cells were washed twice in 10 ml F10P, resuspended in 20 ml of F10P, gassed with 5% CO₂ in air and replaced on the mixer at 37°C for two days. Cell population adjustments were made at 24 and 48 hours to yield a cell population of 0.3×10^6 cells/ml. The cells were then plated in a restrictive media containing 3 ug/ml trifluorothymidine (TFT) which allows TK⁻ cells to grow. Cells were also plated in a non-restrictive media that indicated cell viability.</p> <p>Plates were incubated at 37°C in a humidified 5% CO₂ atmosphere for 10-12 days. Following incubation all plates were scored for total number of colonies/plate. Three counts/plate were made on an automatic colony counter and the median count was recorded. The frequency of mutation by dose was determined by comparing the average number of colonies in the mutagenicity</p>

	<p>plates to the average number of colonies in the corresponding viability plates.</p> <p>For the study to be acceptable the following criteria must be met: mutation frequency of positive controls with or without activation should be twice that of the solvent control; the negative control spontaneous mutation frequency should be in the range of 0.2 to 1.0/10⁴ cells; negative control plating efficiency should be at or above 50%.</p> <p>The following criteria were used as guidelines in judging the significance of test material activity: Positive – if there is a positive dose response and one or more of the three highest dose levels exhibit a mutation frequency two-fold greater than background. Equivocal – no dose response but any one or more dose levels exhibit a 2x increase in mutation frequency over background. Negative - no dose response and none of the dose levels exhibit a 2x increase in mutation frequency over background.</p>
<u>Results</u>	<p>The test substance was not mutagenic in this assay in the absence of metabolic activation. The results in the presence of metabolic activation did not permit an accurate assessment of the compounds mutagenic potential.</p>
Remarks	<p>Nonactivated cultures were cloned over a range of concentrations that produced 2 to 92% total growth. The S-9 cultures were cloned over a range of test article concentrations that produced 6 to 125% total growth.</p> <p>Two of the nonactivated cultures (0.013 and 0.010 ul/ml) that were cloned exhibited mutation frequencies that were 10.8 and 2.5X the mean mutation frequency of the solvent controls. The total growth of these cultures was 2 and 4%. None of the remaining cultures exhibited mutation frequencies that were significantly greater than the mean mutant frequency of the solvent controls. The total growth of these cultures ranged from 20 to 92%. The Study Director did not consider these two cultures significant since mutation frequencies observed at such highly toxic levels may be due to epigenetic events.</p> <p>The +S9 culture treated at 0.024 ul/ml exhibited a mutation frequency that was 7.2 times the mean mutant frequency of the solvent controls. The total growth of this culture was 6%. None of the remaining +S9 cultures that were cloned exhibited mutation frequencies that were significantly greater than the mean mutation frequency of the solvent controls. The total growth of these cultures ranged from 77 to 125%. The total growth of the +S9 treated cultures did not cover the critical range of survival (10-40%) desired for this assay. The test material induced a precipitous toxic response. The cultures treated at the two highest concentrations of test material had 6% and 77% total growth. The Study Director concluded that a repeat assay was not appropriate since the difference in dose concentration between the two highest concentration cultures was only 0.006 ul/ml. The Study Director further concluded that the assay results, in the presence of metabolic activation, did not permit an accurate assessment of the test materials mutagenic potential.</p> <p>Positive and vehicle control group responses were appropriate and met the criteria outlined above.</p>

<u>Conclusions</u>	The test substance was not mutagenic in this assay without metabolic activation. Reliable results were not available in the presence of metabolic activation.
<u>Data Quality</u>	Reliable with restriction (Klimisch Code) Restriction due to the lack of a reliable result in the assay conducted with metabolic activation.
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 4/23/01 (RTA-0)

2.4 Toxicity to Reproduction

Robust Summary 5-ReproTox-1

<u>Test Substance</u>	
CAS #	CAS# 4259-15-8
Chemical Name	2-ethyl hexyl derivative
Remarks	Test material purity not provided.
<u>Method</u>	
Method/Guideline followed	OECD 421
Test Type	Oral reproductive/developmental toxicity screening study
GLP (Y/N)	Y
Year (Study Performed)	1994
Species	Rat
Strain	Sprague-Dawley CD, 71 days of age at initiation of treatment
Route of administration	Orally by gastric intubation
Duration of test	Fo males- 28 days total (14 days premating; 14 day mating period) Fo females- at least 43 days total (14 days premating; mating; 25 days of gestation and 4 days of lactation. F ₁ pups- gestation plus 4 days of lactation.
Doses/concentration levels	0, 30, 100 and 200 mg/kg/day
Vehicle control	Mazola® Corn Oil
Dose volume	5 mL/kg
Sex	Males and females
Frequency of treatment	Once/day, 7 days/week
Analytical confirmation of concentration.	Homogeneity and weekly dose concentration confirmation.
Control and treatment groups	12 Fo rats/sex/group in the control, low, mid and high dose groups.
Post exposure observation period	None
Mating ratio	One male to one female
Duration of mating period	Up to 10 days with initial male; if positive evidence of mating not present (sperm or copulatory plug) then female paired with a second proven breeder male from the same dose group for up to five additional days.
Statistical methods	Pup ratios, pup survival indices, mean number stillborn and dead pups and parental fertility indices were evaluated using the Chi-square test with Yates correction factor. Fo body weights and gains, gestation and lactation body weights and gains, parenteral food consumption, mean litter weights, length of gestation, live litter size and organ weights were evaluated using ANOVA (two-tailed) with Dunnett's test. Histopathological findings were evaluated using the Kolmogorov-Smirnov (one-tailed) test. Data obtained from nongravid animals were excluded from statistical analysis following the mating period. The litter was the experimental unit.
Dose rangefinding study	None

Remarks field for test conditions	<p>All Fo animals were dosed for a minimum of 14 days prior to mating and through the day of necropsy. All animals were examined twice daily for appearance and behavior. Body weights were recorded weekly for both sexes prior to mating; maternal body weights were recorded on gestation days 0, 7, 14 and 20 as well as on lactation days 1 and 4. Food consumption was recorded for corresponding intervals prior to mating, during gestation and lactation. Animals were paired 1:1 for mating. Positive evidence of mating was confirmed by the presence of sperm or a vaginal copulatory plug (day 0 of gestation). If evidence of mating was not present after 10 days, the female was placed with a second male from the same group for 5 days. The second male was a proven breeder based on a prior successful mating. All of the surviving Fo females were allowed to deliver and rear their pups to lactation day 4. The offspring were potentially exposed <i>in utero</i> and through nursing during lactation days 1-4 until euthanization on post-natal day 4. The surviving Fo dams were necropsied on lactation day 4, following at least 43 days of dosing. The surviving Fo males were necropsied after the breeding period, following 28 days of dosing. The Fo females with total litter loss were necropsied within 24 hours. Fo females that failed to deliver were necropsied on post-mating day 25 (with evidence of mating) or 25 days following the termination of the mating period (with no evidence of mating). Organ weights were collected for all Fo animals and microscopic examinations were conducted for all control and high dose animals and for all parental animals not surviving to their scheduled necropsy. Tissues examined microscopically included the epididymides, cervix, coagulation gland, ovaries, pituitary, prostate, seminal vesicles, testes, uterus, vagina vas deferens and gross lesions. Offspring dying between days 0-4 of lactation were necropsied. Carcasses were processed for possible skeletal evaluation. Litters were examined daily. Pups were individually weighed on lactation days 1-4 and were sexed on lactation days 0-4. All surviving pups were euthanized and necropsied on post-natal day 4 with emphasis placed on developmental morphology.</p>
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Results

Fo (Parental Generation)

Two males and three females in the high dose group (200 mg/kg/day) died prior to scheduled necropsy (test days 12, 19, 8, 27 and 39).

These deaths were considered treatment related. Two females in the mid dose group (100 mg/kg/day) and one female in the high dose group were euthanized on lactation days 1 or 2 due to total litter loss. Five of these animals exhibited gastric irritation upon necropsy. All other animals survived to their scheduled sacrifice.

Clinical signs noted in the found dead or sacrificed animals included staining, matting of fur, respiratory distress, hunched appearance and mucoid diarrhea. Clinical findings noted for the surviving mid and high dose males and females included post dosing salivation, brown staining, respiratory distress and diarrhea. No treatment related clinical findings were observed in the low dose (30 mg/kg/day) animals.

Fertility indices (%) for the high dose males and females were slightly lower than control as follows:

	<u>Control</u>	<u>30 mg/kg</u>	<u>100 mg/kg</u>	<u>300 mg/kg</u>
Males	91.7	83.3	83.3	81.8
Females	100	83.3	91.7	81.8

These values were within the range of the test facility historical control data (64-100%). In addition, differences from control were not statistically significant and represent only 1 or 2 fewer successful matings out of 11 or 12 males or females used for mating in the control and high dose groups. A microscopic examination of the reproductive organs of these animals did not reveal any treatment-related effects. The Study Director concluded that the low and mid dose groups were unaffected and that these data did not clearly reflect a treatment related effect in the high dose group. Other reproductive parameters (mating indices, days between pairing and coitus, gestation length and parturition) were unremarkable in all treated groups.

The premating (weeks 1-4) mean body weight gain of the high dose males was statistically significantly reduced compared to control. The mean body weights of the low and mid dose males and all treated female groups were unremarkable during the premating period. Gestation and lactation body weights were unremarkable in all treated groups. Food consumption data were unremarkable in all treated groups (males and females) during the premating, gestation and lactation periods. With the exception of the gastric irritation noted above in several unscheduled deaths, the macroscopic data were unremarkable. Absolute and relative (to body weight) organ weight data as well as the microscopic examination data of the Fo males and females were unremarkable. There were no treatment-related findings evident in any of these data.

F₁ Litter Data

Pup body weights, live litter size and sex ratios were unremarkable. No treatment related effects were evident. An increased number of

	<p>dead pups was noted in the mid dose group on day 0 of lactation. Pup viability indices in the mid dose (lactation days 1 and 4) and high dose (lactation day 4) groups were reduced. This was attributed to total litter loss by three females. Increased pup deaths were observed in the mid and high dose groups during the post-natal period. An increased incidences of pups without milk in the stomach was noted in the mid dose group. No treatment related effects were evident in the necropsy data of these found dead pups or in the necropsy data from scheduled pup necropsies.</p> <p>Chemical analysis of dosing suspensions confirmed that they were homogeneous and of appropriate concentration throughout the study.</p>
Remarks	
<u>Conclusions</u>	<p>Parental (Fo) toxicity was exhibited at dose levels of 100 (mortality, clinical findings) and 200 mg/kg/day (mortality, clinical signs, reduced body weight gain, gastric irritation). A slightly reduced fertility index was also observed at 200 mg/kg/day. No Fo toxicity was observed at 30 mg/kg. Neonatal (F₁) toxicity (mortality) was observed at 100 and 200 mg/kg/day. No F₁ toxicity was observed at 30 mg/kg. Based on these findings the Study Director concluded that the NOAEL for both parental and neonatal toxicity was 30 mg/kg/day.</p>
<u>Data Quality</u>	Reliable without restriction (Klimisch Code)
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 3/14/00 (RTA-030)